UNIVERSITAT POLITÈCNICA DE VALÈNCIA

CORRELATED RESPONSE TO SELECTION FOR LITTER SIZE RESIDUAL VARIANCE IN RABBITS

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This Thesis has been submitted in fulfilment of the requirements for the degree of Doctor at the Universitat Politècnica de València

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ABSTRACT

The thesis is composed for four articles interrelated, where is studied either as relationships between body condition and energetic mobilization in rabbits and as the effect of selection for litter size variability in body condition and energetic mobilization, such as welfare biomarkers in animal production, and in litter size and its components after seven generation of selection.

In this regard, the first article examines the relationships between measures of body condition and energetic mobilization on 157 primiparous rabbit does at mating, delivery and 10 d after delivery, using principal component analysis. Body condition was measured as body weight and perirenal fat thickness. Energetic mobilization was measured as non-esterified fatty acid concentration in blood, before ($NEFA_b$) and after lipolysis stimulation by isoproterenol ($NEFA_r$). All body weights and perirenal fat thickness were located on the first principal component, exhibiting high correlations between them both at the same or different times (from 0.51 to 0.83). All NEFA measurements were located on the second component, showing low correlations with body condition measurements. Both NEFAs showed high positive correlations when measured at the same time (0.65 at mating, 0.72 at delivery and 0.69 at 10 d after delivery), but low correlations when measured at different times (from 0.09 to 0.20).

The second article analyses the correlated response in body condition and fat reserves mobilization in two rabbit lines divergently selected by litter size variability during seven generations of selection. The perirenal fat thickness and the increment in NEFAs from basal concentration until adrenergic stimulation by isoproterenol were measured in 80 females from the high litter size variability line and in 74 females from the low line at second mating, delivery and 10 d after delivery. Data were analysed using Bayesian methodology. Perirenal fat thickness was similar in both lines at mating. However, the high line showed lower fat thickness than the low line at delivery (-0.16 mm, P = 0.86), and this difference remained at 10 d after delivery (-0.17 mm, P = 0.86). Moreover, this line exhibited

30% less concentration in NEFAs at delivery than the low one after adrenergic stimulation by isoproterenol (P = 0.96).

The third and fourth articles study the correlated responses to selection for litter size variability in litter size and its components. A laparoscopy was performed at 12 d of the second gestation on a total of 94 females from the high line and 82 females from the low line, in order to count the ovulation rate (OR) and the number of implanted embryos (IE). The total number of kits born (TNB) and alive (NBA) were also recorded at second parity. Embryonic (ES), fetal (FS) and prenatal (PS) survival were estimated as IE/OR, TNB/IE and TNB/OR, respectively. In the last gestation, 30 non-lactating multiparous does from each line were euthanized at 28, 48 and 72 h of gestation, and embryos were recovered by perfusion of each oviduct and uterine horns. At 28 h of gestation, normal embryos were classified as 2-cell embryos or 4-cell embryos. At 48 h of gestation, normal embryos were classified as early morulae or compacted morulae. At 72 h of gestation, normal embryos were classified as early morulae, compacted morulae or blastocysts. Data were analysed using Bayesian methodology. After seven generations of selection, ovulation rate was similar in both lines. The line selected to reduce the litter size variability showed more embryos at implantation (1.48 embryos, P = 1.00) than the high line. This line also displayed a more advanced embryonic development than the high one from 48 h of gestation, having a lower percentage of early morulae (53.32 % in the low line vs 79.90 % in the high line, P = 0.93) and a higher percentage of compacted morulae (46.87 % in the low line vs 20.29 % in the high line, P = 0.94) at 48 h of gestation, and a lower percentage of early morulae (3.88 % in the low line vs 21.04 % in the high line, P = 0.93) and a higher percentage of blastocysts (62.55 % in the low line vs 51.13 % in the high line, P = 0.71) at 72 h of gestation. A more advanced embryonic development was related to a higher embryonic survival (0.85 in the low line vs 0.78 in the high line, P =1.0). A higher uterine overcrowding of embryos in the low line did not penalise fetal survival, and as a result, this line continued showing a greater number of kits born at birth (+0.98 kits at birth, P = 0.96).

In conclusion, the first study also allowed us to corroborate in rabbits, that body weight and perirenal fat thickness are good predictors of body reserves and both

measurements could be used to estimate energy changes in the mid-long term, while measurements in NEFAs should be used when an accurate measurement of energetic mobilization is needed in short term. The second study shows as a decrease in litter size variability has a favourable effect on body condition and fat reserve mobilization. In this regard, the more homogenous line for litter size seems to adapt better to adverse environments, as it has a greater capacity to mobilize energy reserves at delivery than the heterogeneous line. Besides, the third and fourth studies confirm that selection to reduce litter size variability also has a favourable effect on development of embryo and its survival, showing a higher litter size at birth.

RESUMEN

La tesis se compone de cuatro artículos interrelacionados entre sí, donde se estudia tanto la relación entre la condición corporal y la movilización de energía en la coneja como el efecto de la selección por variabilidad del tamaño de camada en la condición corporal y movilización de reservas energéticas, como biomarcadores del bienestar del animal, y en el tamaño de camada y sus componentes después de siete generaciones de selección.

Concretamente, el primer artículo examina las relaciones entre las medidas de la condición corporal y la movilización de energía en 157 conejas primíparas a la monta, al parto y a los 10 días tras el parto, a través de un análisis de componentes principales. La condición corporal se midió como el peso corporal y el espesor de grasa perirenal. La movilización de energía se midió como la concentración de ácidos grasos no esterificados en sangre, antes ($NEFA_b$) y después de la estimulación lipolítica con isoproterenol ($NEFA_r$). Todos los pesos y espesores de grasa perirenal se situaron sobre la primera componente principal, exhibiendo altas correlaciones entre ellos independientemente del estado fisiológico donde se midieron (de 0.51 a 0.83). Todas las medidas de NEFAs se localizaron sobre la segunda componente principal, mostrando una baja correlación con las medidas de la condición corporal. Los $NEFA_b$ y $NEFA_r$ mostraron elevadas correlaciones entre ellos cuando se midieron en el mismo momento (0.65 a la monta, 0.72 al parto y 0.69 a los 10 días tras el parto), pero bajas correlaciones cuando se midieron en diferentes momentos (de 0.09 a 0.20).

El segundo artículo analiza la respuesta correlacionada sobre la condición corporal y la movilización de reservas grasas en dos líneas de conejas seleccionadas divergentemente por variabilidad del tamaño de camada durante siete generaciones de selección. El espesor de la grasa perirenal y el incremento de los niveles basales de NEFAs después de su estimulación adrenérgica con isoproterenol fueron medidos en 80 hembras de la línea de alta variabilidad y 74 hembras de la línea de baja variabilidad a la segunda monta, al parto y a los 10 días tras el parto. Los datos fueron analizados utilizando metodología Bayesiana. El espesor de la grasa

perirenal fue similar en ambas líneas a la monta. Sin embargo la línea de alta mostró un menor espesor de grasa que la línea de baja al parto (-0.16 mm, P = 0.86), y esta diferencia se mantuvo a los 10 días después del parto (-0.17 mm, P = 0.86). Por otro lado, esta línea exhibió un 30% menos de NEFAs al parto que la línea de baja tras la estimulación adrenérgica con isoproterenol (P = 0.96).

El tercero y cuarto artículo estudian la respuesta correlacionada de la selección por variabilidad del tamaño de camada sobre el tamaño de camada y sus componentes. Se realizó una laparoscopía a los 12 días de la segunda gestación en un total de 94 hembras de la línea de alta y 82 hembras de la línea de baja para estimar la tasa de ovulación (OR) y el número de embriones implantados (IE). Se contabilizó el número de gazapos nacidos totales (TNB) y vivos (NBA) al segundo parto. La supervivencia embrionaria (ES), fetal (FS) y prenatal (PS) fueron estimadas como IE/OR, TNB/IE y TNB/OR, respectivamente. En la última gestación, se sacrificaron 30 hembras no lactantes en cada una de las líneas a 28, 48 y 72 horas de gestación, y los embriones fueron recuperados tras la perfusión de los oviductos y sus correspondientes cuernos uterinos. A las 28 horas de gestación, los embriones recuperados fueron clasificados en un estado de desarrollo de 2 o 4 células. A 48 horas de gestación, los embriones recuperados fueron clasificados como mórulas tempranas o compactas. A 72 horas de gestación, los embriones recuperados fueron clasificados como mórulas tempranas, mórulas compactas o blastocitos. Los datos fueron analizados utilizando metodología Bayesiana. Después de siete generaciones de selección, la tasa de ovulación fue similar en ambas líneas. La línea seleccionada para reducir la variabilidad en tamaño de camada mostró un mayor número de embriones implantados (1.23 embriones, P = 1.00) que la línea de alta. También, esta línea mostró un desarrollo de los embriones más avanzado que la línea de alta a partir de las 48 horas de gestación, exhibiendo un menor porcentaje de mórulas tempranas (53.32 % en la línea de baja vs 79.90 % en la línea de alta, P = 0.93) y un mayor porcentaje de mórulas compactas (46.87 % en la línea de baja vs 20.29 % en la línea de alta, P = 0.94) a 48 horas de gestación, y un menor porcentaje de mórulas tempranas (3.88 % en la línea de baja vs 21.04 % in la línea de alta, P = 0.93) y un mayor porcentaje de blastocitos (62.55 % en la línea de baja vs 51.13 % en la línea de alta, P

= 0.71) a 72 horas de gestación. Un desarrollo más avanzado del embrión está relacionado con una mayor supervivencia de éste (0.85 en la línea de baja vs 0.78 en la línea de alta, P =1.0). Por otro lado, un mayor atestamiento de embriones en el útero de la línea de baja variabilidad no penalizó la supervivencia fetal, y como resultado, esta línea continuó mostrando un mayor número gazapos al parto (+0.98 gazapos al parto, P = 0.96).

En conclusión, el primer estudio nos permite corroborar también en conejo, que el peso y el espesor de grasa perirenal son buenos predictores de las reservas corporales y que ambas medidas podrían usarse para estimar los cambios energéticos a medio plazo, mientras que las medidas de NEFAs se deberían usar cuando se necesita una medida precisa de la movilización de reservas energéticas a corto plazo. El segundo estudio muestra como disminuir la variabilidad del tamaño de camada tiene un efecto favorable sobre la condición corporal y la movilización de reservas grasas. En este sentido, la línea más homogénea para el tamaño de camada parece adaptarse mejor a ambientes adversos, al mostrar una mayor capacidad de movilizar las reservas corporales al parto que la línea heterogénea. Por otro lado, el tercer y cuarto estudio confirman que la selección para reducir la variabilidad del tamaño de camada tiene también un efecto favorable sobre el desarrollo del embrión y su supervivencia, mostrando un mayor tamaño de camada al parto.

RESUM

La tesi es compon de quatre articles interrelacionats entre si, on s'estudia l'efecte de la selecció per variabilitat de la grandària de ventrada en la condició corporal i mobilització de reserves energètiques, com biomarcadores del benestar de l'animal, i en la grandària de la ventrada i els seus components després de set generacions de selecció.

Concretament, el primer article examina les relacions entre les mesures de la condició corporal i la mobilització d'energia en 157 conilles primípares a la munta, al part i als 10 dies després del part, usant una anàlisi de components principals. La condició corporal es va mesurar com el pes corporal i la grossària de greix perirenal. La mobilització d'energia es va mesurar com la concentració d'àcids grassos no esterificats basals (NEFAb) i després de l'estimulació lipolítica amb isoproterenol (NEFAr). Tots els pesos i grossàries de greix perirenal es van situar sobre la primera component principal, exhibint altes correlacions entre ells al mateix moment o en distints moments fisiològics (de 0.51 a 0.83). Totes les mesures de NEFA es van localitzar sobre la segona component principal, mostrant una baixa correlació amb les mesures de la condició corporal. Els NEFAb i NEFAr van mostrar elevades correlacions quan es van mesurar en el mateix moment (0.65 a la munta, 0.72 al part i 0.69 als 10 dies després del part), però baixes correlacions quan es van mesurar en diferents moments (de 0.09 a 0.20).

El segon article analitza la resposta correlacionada sobre la condició corporal i la mobilització de reserves greixos en dos línies de conills seleccionats divergentment per variabilitat de la grandària de la ventrada durant set generacions de selecció. La grossària del greix perirenal i l'increment dels nivells basals de NEFAs després de la seua estimulació adrenérgica amb isoproterenol van ser mesurats en 80 femelles de la línia d'alta i 74 femelles de la línia de baixa a la segona munta, al part i als 10 dies després del part. Les dades van ser analitzats amb metodologia Bayesiana. La grossària del greix perirenal va ser semblant en ambdós línies. No obstant això la línia d'alta va mostrar una menor grossària de greix que la línia de baixa al part (-0.16 mm, P = 0.86), i esta diferència es va mantindre als 10 dies després del part (-

0.17 mm, P = 0.86). D'altra banda, esta línea va exhibir un 30% menys de NEFAs al part que la línea de baixa després de l'estimulació adrenérgica amb isoproterenol (P=0.96).

El tercer i quart articles estudien la resposta correlacionada de la selecció per variabilitat de la grandària de la ventrada sobre el grandària de la ventrada i els seus components. Es va realitzar una laparoscopía als 12 dies de la segona gestació en un total de 94 femelles de la línia d'alta i 82 femelles de la línia de baixa per a estimar la taxa d'ovulació (OR) i el nombre d'embrions implantats (IE). Es comptabilitze el número de nascuts totals (TNB) i vius (NBA) al segon part. La supervivència embrionària (ES), fetal (FS) i prenatal (PS) van ser estimades com IE/OR, TNB/IE i TNB/OR, respectivament. En l'última gestació, es va practicar una eutanàsia a 30 femelles no lactants en cada una de les línies d'alta i de baixa variabilitat en grandària de la ventrada a 28, 48 i 72 hores de gestació, i els embrions van ser recuperats després de la perfusió de cada oviducte i part de l´úter corresponent. A les 28 hores de gestació, els embrions recuperats van ser classificats en un estat de desenrotllament de 2 o 4 cèl·lules. A 48 hores de gestació, els embrions recuperats van ser classificats com mórulas primerenques o compactes. A 72 hores de gestació, els embrions recuperats van ser classificats com mórulas primerenques, mórulas compactes o blastòcits. Les dades van ser analitzats utilitzant metodologia Bayesiana. Després de set generacions de selecció, la taxa d'ovulació va ser semblant en ambdós línies. La línia seleccionada per a reduir la variabilitat en grandària de la ventrada va mostrar un número més gran d'embrions implantats (P = 1.00) que la línia d'alta. També, esta línia va mostrar un desenrotllament dels embrions més avançat que la línia d'alta a partir de les 48 hores de gestació, exhibint un menor percentatge de mórulas primerenques (53.32 % en la línia de baixa vs 79.90 % en la línia d'alta, P = 0.93) i un major percentatge de mórulas compactes (46.87 % en la línia de baixa vs 20.29 % en la línia d'alta, P = 0.94) a 48 hores de gestació, i un menor percentatge de mórulas primerenques (3.88 % en la línia de baixa vs 21.04 % in la línia d'alta, P = 0.93) i un major percentatge de blastòcits (62.55 % en la línia de baixa vs 51.13 % en la línia d'alta, P = 0.71) a 72 hores de gestació. Un desenrotllament més avançat de l'embrió està relacionat amb una major

supervivència d'este (0.85 en la línia de baixa vs 0.78 en la línia d'alta, P =1.0). D'altra banda, un major atapeïment d'embrions en l'úter de la línia de baixa no va penalitzar la supervivència fetal, i com resultat, esta línia va continuar mostrant un número més gran nascuts al part (+0.98 nascuts al part, P = 0.96).

En conclusió, el primer estudi ens permet corroborar també en conill, que el pes i la grossària de greix perirenal són bons predictors de les reserves corporals i que ambdós mesures podrien estimar els canvis energètics a mitjà termini, mentres que les mesures de NEFAs s'haurien d'usar quan es necessita una mesura precisa de la mobilització de reserves energètiques a curt termini. El segon estudi mostra com disminuir la variabilitat de la grandària de la ventrada té un efecte sobre la condició corporal i la mobilització de reserves greixos. En este sentit, la línia més homogènia per a la grandària de la ventrada pareix adaptar-se millor a ambients adversos, al mostrar una major capacitat de mobilitzar les reserves corporals al part que la línia heterogènia. D'altra banda, el tercer i quart estudi confirmen que la selecció per a reduir la variabilitat de la grandària de la ventrada té també un efecte favorable sobre el desenrotllament de l'embrió i la seua supervivència, tendint a una major grandària de la ventrada al part.

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Chapter 1

GENERAL INTRODUCTION

1.1 The genetic improvement in meat rabbits

The existence of breeding programmes has had an important role to improve efficiency in meat rabbit production, which has allowed that this sector has become an intensive farming industry and similar to swine or poultry. The breeding goals in an animal breeding programme are commonly established according to the economic importance of the traits. To our knowledge, there are only 4 former studies on economic weights in rabbits, two in Spanish industry (Armero and Blasco, 1992; Cartuche *et al*., 2014), one in Australian industry (Prayaga and Eady, 2000) and one in French industry (Eady and Garreau, 2008) under restricted feeding. Number of litters per doe and year showed the highest economic weight within reproductive traits, and followed closely by litter size regardless of the country under study, while food conversion rate was the trait with highest economic weight within growth traits (Table 1.1). Number of litters per doe and year is a trait with a high economic weight; however, it must be noted that this trait depends directly on kindling interval and female fertility that have a large management component (Ragab, 2012; Piles *et al.,* 2005). Therefore number of litters per doe and year is a not a sufficiently heritable trait and with sufficient genetic variation to expect an important progress in traditional selection methods, losing prominence in favour of litter size. Although, there have been great changes in the economic weights during these last 22 years, the economic weights for litter size and feed conversion rate were reduced near half; it must stress that both traits are still the most important traits in rabbit meat production (see Table 1.1). When economic weights are estimated in a context of restricted feeding, for a better control of enterocolitis (Boisot *et al.,* 2003), litter size showed the highest economic weight (Eady and Garreau, 2008). Average daily gain and feed conversion rate had high and similar economic weights (Eady and Garreau, 2008), due that using restricted feeding will reduce feeding costs more than feeding *ad libitum*.

All these results explain why intensive meat rabbit production is based on the three-way crossbreeding scheme. The hybrid doe comes from the cross of two maternal lines in order to exploit heterosis and complementarity of the maternal traits

(Baselga, 2004). Maternal lines are selected for litter size at birth or at weaning (Lebas *et al*., 1997; Piles *et al*., 2006a, Ragab and Baselga, 2011), and terminal sire lines were selected for improvement the food efficiency through selection for growth rate post-weaning or for body weight at a point close to market age (Rochambeau *et al*., 1989; Lukefahr *et al*., 1996; Piles and Blasco, 2003; Larzul *et al*., 2005). These growth traits are easier and cheaper to record than feed conversion index, and have a favourable genetic correlation with it (Piles *et al*., 2004), which is a very important productive cost.

Recently, others functional traits are emerging successfully as criteria in breeding programmes, either in maternal lines such as the length of does' productive lifes, ovulation rate and kit survival (Garreau *et al.,* 2008a; Piles *et al.,* 2006b; Sánchez *et al*., 2008; Laborda *et al*., 2011, Ziadi *et al.,* 2013; Larzul *et al*., 2014) or in paternal lines such as carcass dressing percentage, thigh muscle volume, intramuscular fat, heat tolerance, resistance to pasteurellosis, and diseases causing digestive disorders (Eady *et al*., 2007; Garreau *et al*., 2008b; Zomeño *et al*., 2013: Sánchez and Piles, 2013; Matics *et al.,* 2014).

The future priorities in rabbit breeding would be related to improvement of the safety of rabbit products and animal welfare, through resistance to disease and stress (robustness or resiliency), which leading to better female's adaptation to changing environmental conditions.

Table 1.1. Absolute (EW) and relative (REW) economic weights of the main traits of the profit function in €/unit of the trait.

		Armero and Blasco (1992)		Prayaga and Eady (2000)		Cartuche *et al.* (2014)	Eady and Garreau (2008)[a]	
Traits	Unit	EW	EW[1]	EW	EW[2]	EW	REW	REW[3]
. Reproductive traits								
Pregnancy rate	increase by 1 %					1.72		
Litter size	increase by 1	16.90	30.46	15.03	22.44	15.66	45.52	48.82
Number of litter per doe and year	increase by 1	21.83	39.34	16.37	24.44			
Lactation survival	increase by 1%	1.96	3.53	1.70	2.54	1.71		
Fattening survival	increase by 1%	2.30	4.15	1.93	2.89	1.96		
Replacement rate of the farm per doe and year	increase by 1%	-0.45	-0.81	-0.23	-0.34	-0.29		
. Growth traits								
Daily feed intake during lactation	decreasea by 1 g/d	0.52	0.90	0.40	0.59			
Daily gain during lactation	increase by 1 g/d	0.38	0.68	0.21	0.32			
Daily feed intake during fattening	decreasea by g/d	0.41	0.72	0.49	0.72	0.50		
Daily gain during fattening	increase by 1 g/d	1.53	2.70	1.23	1.84	1.33	11.82	12.68
Feed conversion rate during fattening	decrease by 0.1 g/g	18.80	33.88			20.19	10.26	11.01
. Healthy								
Resistance to enterocolitis							4.41	4.73

[a] Eady and Garreau (2008) estimated the relative economic weights (REW) in a context of restricted feeding. [1] Economic weight according to Armero and Blasco (1992) adjusted to constant Euros (Base 100=2014). [2] Economic weights according to Prayaga and Eady (2000) adjusted to constant Euros (Base 100=2014). [3] Relative economic weights according to Eady and Garreau (2008) adjusted to constant Euros (Base 100=2014).

1.2 Environmental sensitivity and its impact on animal breeding

The aim of the genetic selection in animal breeding has traditionally been to increase (or decrease) the mean of the productive traits. Overall, this intensive selection to increase productivity has had success but it has also had negative consequences on behaviour and welfare in animals, causing an increase in eliminating farm animals at early age (Rauw *et al.,* 1998). For this reason, welfare will probably play an important role in future breeding goals for domestic animals, being included already in several breeding programs (e.g., lameness in dairy cows and faecal egg count in sheep, see Rodenburg and Turner, 2012).

Animal welfare is related with a good health and a low stress response (Carenzi and Verga, 2009), and in a consequence, it is linking to good adaptation or less sensitivity of animal to environmental effects (Mormede and Terenina, 2012). Robustness and resiliency are terms that are being used frequently in related to adaptation to environment, although there are slight differences. Robustness is a property that allows an animal to maintain its functions despite external and internal perturbations (Kitaro, 2004); while resilience is defined as the ability to maintain critical functionality across different possible states and can gradually return to its equilibrium state, that is it can survive large perturbations through adaptation and evolution (Fiksel, 2003). Both robustness and resilience refer to the ability of a system to survive disruptions. However, robustness is considered a static concept where the system can resist disruptions and retain its previous stable situation, whereas, resilience is more of a dynamic concept incorporating adaptation where a system can return to a new stable situation after surviving a threat (see figure 1.1). Therefore, resilience is also related to plasticity. Note that if the environmental pressures are too high, the stabilizing mechanisms can fail, functionality breaks down and the characteristic of robustness does not recover, as Veerkamp *et al.* (2009) reported in cattle. The adaptation to environmental changes can be measured indirectly through to uniformity in productivity along lifespan of animal. Therefore, increase uniformity could be a useful tool to improve animal welfare.

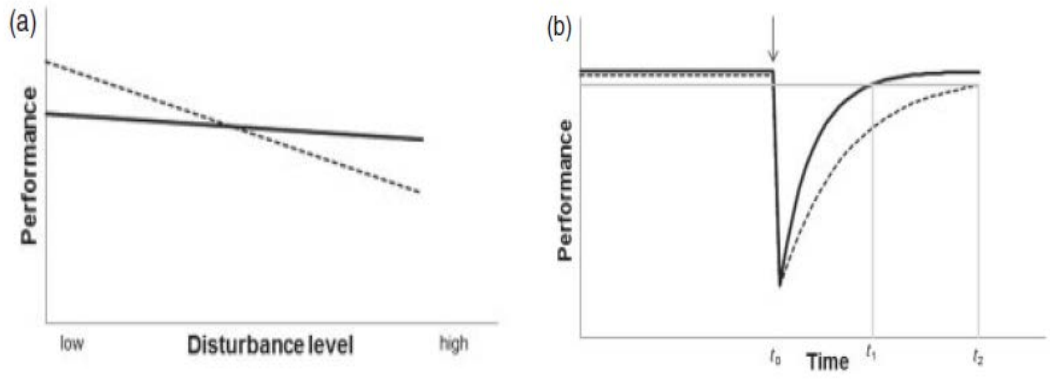

Figure 1.1 a) Robustness as the ability to perform under high disturbance levels. The animal represented by the dashed line is less robust than the animal shown by the solid line. b) Resilience as the ability of an animal to bounce back after disturbance at time t_0. One animal (solid line) is able to recover more quickly, i.e. in this case reach an arbitrary level of 95% of the pre-disturbance performance at t_1, than the other animal (dashed line), which reaches the recovery point later (at t_2) (taken from Döring et al., 2015).

Besides, the uniformity in animal production is an economically interesting trait for breeders. In prolific species, the uniformity in litter size facilitates management by reducing fostering. The homogeneity in birth weight within litter is also an important trait in prolific species like rabbits (Bolet *et al.,* 2007) and pigs (Berard *et al.,* 2008), because increasing weight homogeneity within the litter reduces the competition between littermates and increases the viability of them (Garreau *et al.,* 2008a in rabbits; Damgaard *et al.,* 2003 in pigs). Optimal weights at slaughtering for pigs, broilers and lambs are demanded by industry. The profits of the breeders depend on their ability to send large homogenous groups to the slaughterhouse that penalizes carcasses outside of standard range (Kanis *et al.,* 2006). Also beef industry is interested to improve the uniformity of production traits such as carcass weight, fat deposition and carcass composition and pH 24 h after slaughter (Mach *et al.,* 2008).

Moreover, another interesting aspect of reducing environmental variability is that can augment the heritability of the selected traits (Formoso-Rafferty *et al.,* 2017), facilitating selection in traits with very low heritability, such as litter size (Argente *et al.*, 2010).

1.3 Genetic control of environmental variability

Different strategies can be used to reduce variability, e.g. management and selection, but selection can be effective only when there are genetic differences among animals in phenotypic variability. After correcting data by environmental effects, the remaining environmental variance is the residual variance caused by non-controlled random effects. There is evidence that residual variance is under genetic control in several species. Most of this evidence is indirect because it comes from data bases analyses and not from experiments designed to find the genetic determination of the residual variance. Tables 1.2 and 1.3 show residual variance heritability and genetic coefficients of variation for litter size (San Cristobal-Gaudy *et al.,* 2001, in sheep; Sorensen and Waagepetersen, 2003 and Sell-Kubiak *et al.,* 2015a, in pigs; Gutiérrez *et al.,* 2006, in mice), uterine capacity (Ibáñez-Escriche *et al.,* 2008a, in rabbits), pH (San Cristobal-Gaudy *et al*., 1998, in pigs), number of teats (Felleki and Lundeheim, 2015, in pigs), eggshell color (Mulder *et al.,* 2016, in hens), weight at birth (Gutiérrez *et al.,* 2006, in mice; Garreau *et al.,* 2008a, in rabbits; Neves *et al.,* 2011 and Fina *et al.,* 2013 in beef cattle; Sell-Kubiak *et al.,* 2015b, in pigs), weight at slaughter (Rowe *et al.,* 2006, Mulder *et al.,* 2009 and Wolc *et al.,* 2009 in poultry; Ibáñez-Escriche *et al.,* 2008b, in pigs), adult weight (Ros *et al.,* 2004, in snails; Janhunen *et al.,* 2012 and Sae-Lim *et al.,* 2015, in rainbow trout; Sonesson *et al.,* 2013, in Atlantic salmon; Marjanovic *et al.,* 2016, in Nile tilapia), conformation (Wolc *et al.,* 2009, in broiler; Marjanovic *et al.,* 2016 in Nile tilapia), milk yield (Rönnegård *et al.,* 2013 and Vandenplas *et al.,* 2013, in dairy cattle), and milk quality (SanCristobal-Gaudy *et al.,* 1998, on fat/protein rate in goats; Rönnegård *et al.,* 2013 and Vandenplas *et al.,* 2013, on somatic cell score and on saturated and unsaturated fatty acids in dairy cattle).

Table 1.2 Estimates of heritability of residual variance (h^2_v), genetic coefficients of variation (GCV_{Av}) and genetic correlation (ρ) between additive genetic effects for mean and residual variance.

Trait	Specie	h^2_v	GCV_{Av}[a]	ρ	Method[b]	Source
Litter size	*Pigs*	0.026	0.31	-0.62	MCMC	Sorensen and Waagepertersen (2003)
		0.021	0.27	-0.64	MCMC	Yang *et al*. (2011)
		0.012	0.27	0.70	MCMC	Yang *et al*. (2011)[c]
		0.040	0.41	-0.52	REML	Felleki *et al.* (2012)[d]
		0.006	0.09	0.49	REML	Sell-Kubiak *et al.* (2015a)
	Rabbits	0.045	0.42	-0.74	MCMC	Ibáñez-Escriche *et al.* (2008a)[e]
		0.041	0.37	-0.73	MCMC	Yang *et al.* (2011)
		0.017	0.24	0.28	MCMC	Yang *et al.* (2011)[c]
	Mice	0.048	0.44	-0.93	MCMC	Gutiérrez *et al.* (2006)
	Sheep	0.048	0.51	0.19	REML	SanCristobal-Gaudy *et al.* (2001)
Average for litter size		*0.030*	*0.33*	*-0.25*		
pH muscle	*Pigs*	0.039	0.40	0.79	REML	SanCristobal-Gaudy *et al.* (1998)
Number of teats	*Pigs*	0.060	0.48	0.80	REML	Felleki and Lundeheim (2015)
Eggshell color	*Hens*					
	(purebred)	0.010	0.28	-0.06	REML	Mulder *et al.* (2016)
	(crossbred)	0.011	0.26	0.43	REML	Mulder *et al*. (2016)
Milk yield	*Dairy*	0.003	0.25	0.60	REML	Rönnegård *et al.* (2013)
		0.002	0.17	0.47	REML	Vandenplas *et al.* (2013)
Fat/protein	*Goats*	0.000	0.00	-	REML	SanCristobal-Gaudy *et al*. (1998)
Somatic cell score	*Dairy*	0.006	0.26	0.38	REML	Rönnegård *et al.* (2013)
		0.003	0.16	0.27	REML	Vandenplas *et al.* (2013)
Saturated fatty acids	*Dairy*	0.001	0.12	0.28	REML	Vandenplas *et al.* (2013)
Unsaturated fatty acids	*Dairy*	0.003	0.12	0.24	REML	Vandenplas *et al.* (2013)
C18:1 cis-9 contents	*Dairy*	0.004	0.12	0.22	REML	Vandenplas *et al.* (2013)
Average for yield and quality milk		*0.003*	*0.15*	*0.23*		

[a] $GCV_{Av} = \sigma_{AV}/\sigma^2_E$, where σ_{AV} is the genetic standard deviation in the residual variance and σ^2_E is the mean residual variance a measure of evolvability (Houle 1992). [b] Methods classified into analysis of variance (ANOVA), residual maximum likelihood (REML) and Markov chain Monte Carlo (MCMC). [c] after Box–Cox transformation of data. [d] using the same data base for Sorensen and Waagepertersen (2003). [e] analysed trait was uterine capacity, highly correlated to litter size.

Table 1.3 Estimates of heritability of residual variance (h^2_v), genetic coefficients of variation (GCV_{Av}) and genetic correlation (ρ) between additive genetic effects for mean and residual variance (continuation of Table 1.2).

Trait	Specie	h^2_v	GCV_{Av}[a]	ρ	Method[b]	Source
Body weight at birth	*Pigs*					
	(Large White)	0.008	0.10	0.62	REML	Sell-Kubiak *et al.* (2015b)
	(Landrace)	0.011	0.11	0.55	REML	Sell-Kubiak *et al.* (2015b)
	Rabbits	0.014	0.25	-	REML	Garreau *et al.* (2008a)
	Mice	0.208	0.21	0.97	MCMC	Gutiérrez *et al.* (2006)
		0.039	0.37	-0.81	MCMC	Gutiérrez *et al.* (2006)[c]
		0.006	0.36	-0.31	MCMC	Ibáñez-Escriche *et al.* (2008c)[d]
	Beef	0.094	0.69	0.42	REML	Neves *et al.* (2011)
		0.130	-	0.44	MCMC	Fina *et al.* (2013)
Body weight at slaughter	*Pigs*	0.011	0.34	-0.07	MCMC	Ibáñez-Escriche *et al.* (2008b)
	Broiler (male)	0.029	0.30	-0.17	ANOVA	Rowe *et al.* (2006)
		0.046	0.44	-0.45	REML	Mulder *et al.* (2009)
		0.030	0.32	-0.23	REML	Wolc *et al.* (2009)
	Broiler (female)	0.031	0.32	-0.11	ANOVA	Rowe *et al.* (2006)
		0.047	0.57	-0.41	REML	Mulder *et al.* (2009)
		0.038	0.37	-0.22	REML	Wolc *et al.* (2009)
Adult body weight	*Snails*	0.017	0.58	0.81	MCMC	Ros *et al.* (2004)
	Rainbow trout	0.024	0.38	-0.16	REML	Janhunen *et al.* (2012)
		0.011	0.21	0.30	REML	Sae-Lim *et al.* (2015)[e]
		0.010	0.19	0.79	REML	Sae-Lim *et al.* (2015)[f]
	Atlantic salmon	0.030	0.41	-	REML	Sonesson *et al.* (2013)
	Nile tilapia	0.021	0.58	0.58	REML	Marjanovic *et al.* (2016)
Weight gain	*Mice*	0.018	0.47	-0.19	MCMC	Ibáñez-Escriche *et al.* (2008c)[g]
	Beef	0.020	0.23	-0.02	REML	Neves *et al.* (2011)[h]
		0.012	0.18	-0.09	REML	Neves *et al.* (2011)[i]
Conformation score	*Broiler (male)*	0.023	0.25	0.21	REML	Wolc *et al.* (2009)
	Broiler (female)	0.032	0.31	0.20	REML	Wolc *et al.* (2009)
	Beef	0.019	0.26	0.17	REML	Neves *et al.* (2011)[j]
		0.006	0.15	0.06	REML	Neves *et al.* (2011)[k]
Morphological traits	*Nile tilapia*	0.009	0.39	0.11	REML	Marjanovic *et al.* (2016)[l]
		0.012	0.42	0.37	REML	Marjanovic *et al.* (2016)[m]
		0.014	0.45	0.20	REML	Marjanovic *et al.* (2016)[n]
Average body traits		*0.033*	*0.34*	*0.12*		

[a] $GCV_{Av} = \sigma_{AV}/\sigma^2_E$, where σ_{AV} is the genetic standard deviation in the residual variance and σ^2_E is the mean residual variance a measure of evolvability (Houle 1992). [b] Methods classified into analysis of variance (ANOVA), residual maximum likelihood (REML) and Markov chain Monte Carlo (MCMC). [c] litter weight at birth. [d] body weight at 21 d. [e] in the selection nucleus. [f] in the sea. [g] weight gain from 21 to 42 days. [h] weight gain from birth to weaning. [i] weight gain from weaning to yearling. [J] at weaning. [k] at year. [j, m, n] length, depth and width in Nile tilapia.

The reproductive and growth traits show low and similar average values for residual variance heritability (0.03), but genetic standard deviations exhibit high average values for both groups of traits (0.33 in litter size vs. 0.34 in growth traits). The low values of residual variance heritability show that a large amount of information is necessary to estimate accurately aditive value for enviromental variability, but the high values of genetic standard deviations indicate that there are opportunities to reduce variability, i.e. to improve uniformity, by selection. The scenario is completely different to milk yield and quality, whose residual environmental heritability and genetic coefficient of variation are extremely small (0.003 and 0.15, respectively) to expect to succeed in selection.

1.4 Relationship between mean and variability

Animal production has interests in reducing the variance but without reducing its possibilities for improving the mean. Decreasing variability can affect the mean of the trait, but it will depend on genetic correlation between both traits. Several authors have estimated the genetic correlation between the genetic effects of the mean and the genetic effects of variability (see Table 1.2 and Table 1.3).

No genetic correlations were found between mean and residual variance by several authors. For example, no correlation between mean and residual variance was reported for the ratio of fat to protein contents in goats (San Cristobal-Gaudy *et al.,* 1998), slaughter weight in pigs (Ibáñez-Escriche *et al.,* 2008b), and weight gain and conformation score in cattle (Neves *et al.,* 2011). Besides, selection by litter variability of birth weight did not modify its mean in rabbits (Garreau *et al.,* 2008a).

Other authors found positive genetic correlations between mean and residual variance for body weight at birth (Sell-Kubiak *et al.,* 2015b, in pigs; Gutiérrez *et al.,* 2006, in mice; Neves *et al.,* 2011 and Fina *et al.,* 2013*,* in Nellore and Bruna dels Pirineus beef cattle, respectively), adult body weight (Ros *et al.,* 2004, in *Helix aspersa* snails; Sae-Lim *et al.,* 2016, in Rainbow trout; Marjanovic *et al.,* 2016, in Nile tilapia), body condition (Wolc *et al.,* 2009, in broiler; Marjoanovic *et al.,* 2016, in Nile tilapia), pH (SanCristobal-Gaudy *et al.,* 1998, in pigs), number of teats in pigs (Felleki

and Lundeheim, 2015), and milk yield and quality in dairy cattle (Rönnegård *et al.,* 2013; Vandenplas *et al.,* 2013).

Different results were shown by Sorensen and Waagepetersen (2003), who detected a negative genetic correlation between mean and residual variance in pig litter size data, and it was confirmed by Felleki *et al.* (2012) with the same dataset using other methodologies. Ibáñez-Escriche *et al.* (2008a) estimated the correlation between the genetic effects of the mean and those on the residual variance in uterine capacity in rabbits, and it was -0.74. Uterine capacity is highly correlated to litter size (Argente *et al.,* 2000). In mice, a strong negative genetic correlation was found between mean and residual variance for litter size (-0.93) and litter weight at birth (-0.82) by Gutiérrez *et al.* (2006). Genetic correlations between mean and residual variance for body weight was also negative in broiler chickens (Rowe *et al*., 2006; Wolc *et al*., 2009; Mulder *et al.,* 2009). Note that these correlations are being questioned, due to they were obtained using highly parameterized and not robust models. For example, Yang *et al.* (2011) showed that small deviations from normality in the residuals can substantially change the genetic parameters estimated. Therefore, it must be carried out well-designed selection experiments, in order to validate the estimated genetic parameters for interested traits.

1.5 Selection experiments for environmental variability

Other evidence of the existence of a genetic component in environmental variance comes from some experiments using inbred lines in *Drosophila melanogaster* (Morgante *et al.,* 2015) and domestic species like rabbits, mice and pigs. In particular, it has been perfomed five selection experiments for environmental variability (Table 1.4); three for birth weight (Bodin *et al.,* 2010a in rabbits; Pun *et al.,* 2013 and Formoso-Rafferty *et al.,* 2016 in mice), one for *Semimembranosus* ultimate pH (Larzul *et al.,* 2006 in pigs), and one for litter size (Argente *et al.,* 2014a in rabbits). Divergent selection was performed in all experiments, with a line to increase the variability of character (H line) and another line to decrease the variability (L line). When the criterion of selection of the lines is to increase or reduce the variability around an optimum, the canalizing selection is applied, and

this is the criterion used in the INRA experiments of pH in pigs and birth weight in rabbits. The heteroscedastic model, developed by SanCristobal-Gaudy *et al.* (1998), was applied in all the experiments, except that carried out by Argente *et al.* (2014a) for litter size variability at birth in rabbits. The heteroscedastic model assumed that the environmental variance is heterogeneous and partially under genetic control. For the selection experiment on litter size variability in rabbits, it is the first experiment in which selection has been directly performed on environmental variance, treating it as an observed trait.

One of the first canalizing experiments was the selection of *Semimembranosus* ultimate pH in pigs (Larzul *et al.,* 2006). Direct response (5.69 in both lines) and correlated response in *Abductor* and *Longissimus dorsi* were not observed, but estimated breeding values were based on only four progeny and had low accuracy. Nevertheless, there was correlated response in pH of *Gluteus superficialis*, showing higher value the L line than the H line (5.63 vs 5.58, respectively). Moreover, the H line was leaner than L line (61.3 and 60.0 for lean content, 20.8 and 23.5 mm for backfat thickness).

Another canalizing selection experiment based on the homogeneity of birth weight in rabbits was carried out at the INRA (Garreau *et al.,* 2008a; Bodin *et al.,* 2010a,b). The difference of within-litter birth weight standard deviation between the two lines was 0.61 g (9.17 g in the H line and 8.56 g in the L line) in the first generation, but this difference remained almost constant until the generation 4 (Garreau *et al.,* 2008a). Moreover, there was no correlated response for the individual weight at birth or weaning. Only after generation 5 was further response achieved in the experiment (Bodin *et al.*, 2010a). After 10 generations, the standard deviation was 11.26 g in the H line and 7.34 g in the L line. The correlated response in the mortality at lactation was 32.7% and 17.7% and in the H and L lines, respectively.

In mice, Pun *et al.* (2013) performed a divergent selection experiment for environmental variability of the birth weight. However, this experiment failed after 10 generations of selection, because as the authors argued, the trait was attributed to the individual when it should have been assigned to the mother (Pun *et al.,* 2013). Moreover, they also identified some anomalous results such as an extreme genetic correlation between the birth trait and its environmental variability, or a too high

value for the additive genetic variance of the environmental variability as first warned by Hill and Mulder (2010). After that, this team returned to start a selection experiment for variability in birth weight where the selection criterion was the predicted breeding value for birth weight environmental variability associated with the mother (Formoso-Rafferty *et al.,* 2016). At the seventh generation of selection, the means were 0.037 g^2 and 0.014 g^2 for variance of birth weight, 0.17 g and 0.11 g for standard deviation of birth weight, and 1.64 g and 1.47 g for body weight, in the H and the L lines, respectively.

Table 1.4 Description of selection experiments for environmental variance.

Trait	Species	Ge	Nº per line		Method	R	Source
			♀	♂			
pH muscle	*Pigs*	4	25-35	4	HM	0.00	Larzul *et al.* (2006)
Individual birth weight	*Rabbits*	10	52-68[a]	6-7	HM	3.92[b]	Bodin *et al.* (2010b)
	Mice	10	12	6	HM	0.00	Pun *et al.* (2013)
	Mice	7	43	43	HM	0.02	Formoso-Rafferty *et al.* (2016)[c]
Litter size	*Rabbits*	8	120	25	Direct	1.19	Argente *et al.* (2014a)

Ge generation. R direct response to selection estimated as mean of the marginal posterior distribution of the difference between lines for the selected trait. HM heteroscedastic model developed by San Cristobal-Gaudy *et al.* (1998). [a] in the last generation. [b] difference of within-litter birth weight standard deviation. [c] birth weight was attributed to the dam.

Finally, a selection experiment for environmental variability of litter size is being carried out in rabbits (Argente *et al*., 2014a). In this experiment, the use of complex models on environmental variability is avoided by directly selecting for this trait as an observed trait. Litter size environmental variability was directly recorded by computing the intra-doe variance of litter after correcting litter size for year-season and parity-lactation status. After seven generation of selection, the H and L lines showed a difference of 1.19 $kits^2$ for selection criterion. Besides, the H line showed lower total number of kits born (−0.70 kits) and total number of kits born alive (−0.58 kits) than the L line (Argente *et al.,* 2014a). Indirect response in haematological parameters as immunologic indicators has also been studied,

showing that the L line appears to be more resistant to diseases and more able to withstand adverse environmental conditions (Argente *et al.,* 2014b).

1.6 Body condition and energetic mobilization in animal welfare

Body condition is a common tool for assessing the energy status of dams in animal production. Body condition refers to the state of the body energetic reserves that are used when the females have an energetic demand. It is considered a medium-large measure of energy balance. Body condition is influence by reproductive rhythm (Castellini *et al.,* 2003; Dal Bosco *et al.,* 2003), lactation (Xiccato *et al.,* 2004), reproductive performance (Cardinali *et al.,* 2008) and animal welfare (Rosell *et al.,* 2008). Many methods to evaluate body condition *in vivo* are available in rabbits. Some, such as X-ray tomography (Romvári *et al*., 1998) or imaging by nuclear magnetic resonance (Köver *et al.,* 1998) are useful, but require anaesthesia of animals. The total body electric conductivity (Fortun-Lamothe *et al*., 2002) combines a measurement of body conductivity with the animals' weight to estimate their composition; the advantage is that no preliminary preparation is needed in the animals but the disadvantage is that not provide information about the anatomic distribution of adipose tissue mobilised. The body condition score is easy to apply but it is a subjective method (Cardinali *et al*., 2008). The bioelectrical impedance analysis is based on the determination of differences in the electrical conductivity between the fat and non-fat tissues (Nicodemus *et al.,* 2009). This method assumes a homogeneous distribution in body composition and uniform in cross-sectional area (Arnal *et al.,* 2011). The ultrasound to assess the body composition (Pascual *et al*., 2000) is based on the measurements of the perirenal fat thickness. It is easy to use and it is a direct measurement of variations in the perirenal fat. The perirenal fat is the main adipose tissue, and it is highly correlated with the other adipose tissues (Silva *et al*., 2012). Thus, perirenal fat thickness has been proposed to estimate changes in body condition (Pascual *et al*., 2000).

Negative energy balance is associated with mobilization of body reserves, predominantly localized in fat and muscle tissues (Gross *et al*., 2011). An increase of blood parameters, mainly non-esterified fatty acid (NEFA), generally indicates mobilizations of adipose tissue (Fortun-Lamothe, 2006) to support increased

energy requirements (Gross *et al*., 2013). Therefore, NEFAs show the energy balance in the short term. They are more related to energy balance as feed intake (Brecchia *et al*., 2006), milk yield (Fortun–Lamothe et al., 1999) or heat stress (Savietto *et al*., 2014).

Body condition score and energy management mobility has been proposed as a valid indicator of animal welfare in dairy cows (Roche *et al.,* 2009) and in pigs (Prunier *et al.,* 2010). In rabbits, both body condition and health has been proposed by Rosell and de la Fuente (2008), in order to define doe welfare on commercial farms. Moreover, Theilgaard *et al.* (2007) and Ferrian *et al.* (2013) showed that more robust rabbit females present greater longevity, and better body condition and modulation of the immune system under heat stress conditions when the immune system is effected (Ferrian *et al.,* 2012).

1.7 Litter size and its components in animal welfare

Previously, we have discussed the genetic correlation between the variability of litter size and its mean. Although, it has recently been questioned, large majority of estimates displays a negative correlation between both traits in pigs, mice and rabbits, as shown in table 1.2. Therefore, an increase in litter size variability would be accompanied by a decrease in litter size. It is necessary to highlight that litter size at birth depends on a sequence of reproductive processes as ovulation, number of implanted embryos and survivors at birth.

Litter size is affected by stressful conditions of the mother (Lawlor and Lynch, 2007, in sows; Marai *et al.,* 2002, in rabbits). Besides, some research has shown that maternal stress is associated with lower embryo development and survival (Omtvedt *et al.,* 1971 and Razdan *et al.,* 2002, in sows; Marai et al., 2002, in rabbits; Walsh *et al.,* 2011, in dairy cows; Burkuš *et al.,* 2015, in mice). Our hypothesis is that litter size variability is related to the ability in female to withstand adverse and stressful conditions. Therefore, a decrease in litter size variability would be related to better adaptation in female to adverse and stressful environmental. In a consequence, these females would have a higher embryonic survival, and it leads to larger litter size at birth.

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Chapter 2

OBJECTIVES

In the present thesis, two genetic lines of rabbits selected divergently for litter size variability have been used to know the effect of selection in body condition and energetic mobilization, as welfare biomarkers in animal production, and in reproductive performance after seven generations of selection.

The specific objectives of this thesis are:

Chapter 3. To examine the relationships between measures of body condition and energetic mobilization in rabbit does.

Chapter 4. To evaluate the correlated response in body condition and fat reserves mobilization in two rabbit lines divergently selected by litter size variability during seven generations.

Chapter 5. To analyse correlated response to selection for litter size variability on litter size components after seven generations of selection.

Chapter 6. To assess the effect to selection for litter size variability on early embryonic development and survival after seven generations of selection.

Chapter 3

RELATIONSHIP BETWEEN BODY CONDITION AND ENERGETIC MOBILIZATION IN RABBIT DOES

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ABSTRACT

The present work was performed to examine the relationships between measures of body condition and energetic mobilization in rabbit does. The variables studied were body weight (BW), perirenal fat thickness (PFT), basal non-esterified fatty acid concentration ($NEFA_b$) and non-esterified fatty acids after lipolysis stimulation by isoproterenol ($NEFA_r$). The effect of time of measuring (mating, delivery and 10 d after delivery) was estimated on 157 primiparous does. Correlations between components of body condition were estimated and a principal component analysis performed. The does decreased BW (6%) and PFT (3%), and increased $NEFA_b$ (25%) and $NEFA_r$ (16%) from mating to delivery. Later, $NEFA_b$ and $NEFA_r$ decreased around 20% from delivery to 10 d after delivery without changing perirenal fat thickness. All BW and PFT laid in the first principal component, and all NEFAs laid in the second component, showing low correlations with body condition measurements. Both NEFAs showed high positive correlations when measured at the same time (0.65, 0.72 and 0.69), but low correlations when measured at different times (0.09, to 0.20). We conclude that although body weight and perirenal fat thickness are good predictors of body condition, NEFA should be used when an accurate measurement of energetic mobilization is needed, due to their low correlation.

Keywords: Body condition, NEFA, Perirenal fat thickness, rabbit.

INTRODUCTION

Body condition is a common tool for assessing the energy status of dams in animal production. Body condition refers to the state of the body energetic reserves, i.e. fat deposits, that are used when the does have an energetic demand. Different in vivo techniques have been proposed in order to estimate body condition in rabbits. Total body electric conductivity (Fortun-Lamothe *et al*., 2002), body condition score (Cardinali *et al*., 2008), computer tomography (Romvári *et al*., 1998), bioelectrical impedance analysis (Nicodemus *et al*., 2009), and ultrasound (Pascual *et al*., 2000) have been used to assess body condition. The ultrasound is a simple, low cost and

accurate method to estimate fatty deposits. Perirenal fat is the main adipose tissue and it is highly correlated with the other adipose tissues (Silva *et al*., 2012). Due to this, perirenal fat thickness has been proposed to estimate changes in body condition (Pascual *et al*., 2000).

Negative energy balance is associated with mobilization of body reserves, predominantly localized in fat and muscle tissues (Gross *et al*., 2011). Fortun-Lamothe (2006) indicates that an increase of non-esterified fatty acids (NEFA) concentration in blood generally indicates mobilizations of adipose tissue (Gross *et al*., 2013). An increase in NEFA concentration is interpreted as short-time mobilization, and perirenal fat thickness changes are used to estimate energy changes in the mid-long term. Consequently, body condition and NEFA are both used, as both provide information to interpret properly the energy balance of females (Fortun-Lamothe, 2006).

There are three key moments when the does need to manage their body condition and energetic mobilization; mating (Castellini *et al*. 2006; Brecchia *et al*. 2006), delivery (Rebollar *et al*., 2011; Savietto *et al*., 2016) and early lactation (Quevedo *et al.*, 2006). Our objective was to assess the relationships between body condition and energetic mobilization measurements at these three moments of the reproductive cycle of the doe.

MATERIAL AND METHODS

All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee (Reference number DTA-MJA-001-11), according to Council Directives 98/58/EC and 2010/63/EU.

Animals

One hundred and fifty-seven primiparous dams were used in this study. All animals were reared at the Miguel Hernández University of Elche (Spain). Rabbits were allowed *ad libitum* access to a standard pelleted diet (218 g acid detergent fibre, 174 g crude protein and 11.0 MJ digestible energy, Cunilactal, Nutreco). The does were

kept in individual cages in a farm which had a constant photoperiod of 16 h continuous light: 8 h continuous darkness and controlled ventilation. They were first mated at 18 wk of age and at 10 d after parturition thereafter. If the dams were not receptive, they were mated again a week later. Kits were weaned at 28 d of age. Two synthetic maternal lines were used in the analysis.

Traits

All traits were measured at effective mating, delivery and 10 d after delivery, at the second parity. Dam body weight (BW) was recorded. Does perirenal fat thickness (PFT) was measured by ultrasound imaging as described by Pascual *et al*. (2000), using Justvision 200 SSA-320A Toshiba ultrasound equipment.

Non-esterified fatty acid concentration was determined in basal state ($NEFA_b$) and in response to the adrenergic agent isoproterenol ($NEFA_r$), which increases the lipolysis. Blood was sampled before and 7.5 min after isoproterenol injection (50 µg/kg BW, Sigma 15627). This time interval and concentration of isoproterenol was found appropriate by Theilgaard *et al*. (2005) for assessing the lipolytic potential in rabbits. Blood samples were obtained from the central ear artery at early in the morning hours, before feed was distributed, to prevent the effect of feeding, as proposed by Theilgaard *et al*. (2005). The samples were centrifuged immediately after sampling (4,000 x g, 4 ºC, 15 min) and plasma was stored at -20ºC for further analysis. Plasma NEFA concentrations were determined using the *in vitro* enzymatic colorimetric methodology prepared by the NEFA test Wako C (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Samples were analysed with spectrophotometer UV (Model Hewlett Packard 8453).

Statistical analyses

Differences in body condition and energetic mobilization indicators were estimated with a model including the effects of time of measurement, line, lactation status (lactating or non-lactating at mating), season and dam permanent effect. All analyses were performed using Bayesian methodology. Bounded uniform priors were used for all effects with the exception of the dam permanent effect, considered normally distributed with mean $\mathbf{0}$ and variance $\mathbf{I}\sigma^2_p$. Residuals were a priori normally

distributed with mean **0** and variance $\mathbf{I}\sigma^2_e$. The priors for the variances were also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. Convergence was tested using the Z criterion of Geweke (Sorensen and Gianola, 2002) and Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992). The program Rabbit, developed by Institute for Animal Science and Technology (Valencia, Spain), was used for all procedures.

Correlations between residuals of a model that included line, lactation status and season effects were estimated. A principal component analysis was performed. All these analyses were performed using the SAS statistical package.

RESULTS AND DISCUSSION

Descriptive results of the traits are presented in Table 3.1. Body weight of the females was lower than those reported by Quevedo *et al*. (2006) and Theilgaard *et al*. (2009), but perirenal fat thickness was similar (Quevedo *et al*., 2006) or higher (Theilgaard *et al*., 2009). This may be due to the different feed composition (Quevedo *et al*., 2006) or reproductive rhythm applied (Theilgaard *et al*., 2009). Basal NEFA and NEFA after stimulating lipolysis by injecting isoproterenol showed similar values than Theilgaard *et al*. (2009), and the NEFA levels were also similar to those obtained by Brecchia *et al.* (2006) after 24h of fasting. Both NEFAs showed high variability, with coefficients of variation from 0.40 to 0.47.

Table 3.2 shows the evolution of body condition indicators at the three times in which they were measured. When $|D| > 0$, we consider that there is enough evidence about measurements at different times are different, if the probability of $|D|$ is more than 0.80. Both basal NEFA and NEFA after stimulating lipolysis were higher at delivery than at mating (25% and 16%, respectively), as expected due to higher energetic demand (Rebollar *et al*., 2011) and lower food ingestion of the doe (Pascual *et al*., 2003) at this moment. It is known in dairy cows that during the transition from late gestation to early lactation, considerable amount of adipose tissue is mobilized, resulting in elevated plasma NEFA (Gross et al., 2013). Perirenal

fat thickness and body weight, are in agreement with the NEFA measurements, they are 3% and 6% lower at delivery. Subsequently, both NEFAs were around 20% higher at delivery than at 10 d after delivery, but no differences were found for perirenal fat thickness (D=−0.05, $HPD_{95\%}$= [-0.23, 0.12]). These variations of NEFA concentrations could be due to variation of the flow of NEFA concentration with respect to its oxidation capability and storage (Gross *et al*., 2013), thus this variation is not necessarily attributable to changes in energy balance. From mating to 10 d after delivery, the balance was negative for body weight (92 g) and perirenal fat thickness (0.19 mm). Within a reproductive cycle, the highest value of NEFA was at delivery, which is in agreement with Rebollar *et al.* (2011).

Table 3.1 General mean, standard deviation (SD), coefficient of variation (CV) for measures of body condition and energetic mobilization at mating, delivery and 10 d after delivery.

	Mating			Delivery			10d after delivery		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
BW (g)	3637	368	0.10	3411	413	0.12	3556	457	0.13
PFT (mm)	9.30	0.80	0.09	9.1	0.90	0.09	9.20	1.10	0.10
$NEFA_b$ (mmol/l)	0.53	0.25	0.47	0.66	0.31	0.47	0.53	0.21	0.40
$NEFA_r$ (mmol/l)	0.88	0.39	0.44	1.02	0.36	0.38	0.81	0.32	0.40

BW: body weight. PFT: perirenal fat thickness. $NEFA_b$: basal non-esterified fatty acids concentration. $NEFA_r$: non-esterified fatty acids after lipolysis stimulation.

Table 3.2. Features of the marginal posterior distribution of the difference (D) between body condition and energetic mobilization measurements at different times.

	BW			PFT			$NEFA_b$			$NEFA_r$		
	D	$HPD_{95\%}$	P	D	$HPD_{95\%}$	P	D	$HPD_{95\%}$	P	D	$HPD_{95\%}$	P
Mating - Delivery	227	174, 284	1.00	0.24	0.08, 0.41	1.00	-0.13	-0.20, -0.06	1.00	-0.14	-0.24,-0.05	1.00
Delivery - 10 d after delivery	-135	-192, -77	1.00	-0.05	-0.23, 0.12	0.72	0.13	0.05, 0.20	1.00	0.21	0.10, 0.30	1.00
Mating - 10 d after delivery	92	36, 149	1.00	0.19	0.03, 0.36	0.98	0.00	-0.07, 0.07	0.50	0.07	-0.04, 0.15	0.91

$HPD_{95\%}$: highest posterior density region at 95%. P: probability of the difference being positive when D>0 or negative when D<0. BW: body weight (g). PFT: perirenal fat thickness (mm). $NEFA_b$: basal non-esterified fatty acids concentration (mmol/l). $NEFA_r$: non-esterified fatty acids after lipolysis stimulation (mmol/l).

Table 3.3 shows the coefficients of correlation between traits. In order to facilitate the interpretation of the correlations, we performed a principal components analysis. The first two components explain near 50% of total variation (30% and 19% respectively). Figure 3.1 shows the first and second principal component. All body weights and perirenal fat thickness were located on the first principal component, with the exception of perirenal fat thickness at mating. We found substantial positive correlations between them, both at the same time and at different times (0.51 to 0.83). Body weight and perirenal fat thickness have been proposed as predictors of body reserves by Pascual *et al*. (2000). Both traits are related to energy content, which is highly influenced by the size of the animal. Although high correlations between measurements are expected, some of these correlations are not so high, therefore all of them give useful information about the energy balance in the mid-long term.

All NEFA measurements were located on the second principal component, showing low correlations with the perirenal fat thickness measurements and also with body weight. As NEFA measurements do not depend on body weight or perirenal fat, they show the energy balance in the short term. NEFA should be more related to the energy balance due to differences in feed intake (Brecchia *et al*., 2006), milk yield (Fortun–Lamothe and Prunier, 1999) or heat stress (Savietto *et al*., 2014). Basal NEFA and NEFA after lipolysis stimulation measurements showed high positive correlations when measured at the same time (0.65 to 0.72), indicating that even in a state of high fat mobilization reserves, the does have at any time an important additional capacity for fat reserves mobilization. NEFAs showed low correlations between them when measured at different times (0.09 to 0.20), showing that the actual state of fat reserves mobilization should be measured at each time, being both NEFAs at a time poor predictors of the capacity of reserves mobilization at other times. Xiccato *et al.* (2005) also indicated that NEFA levels in blood did not closely reflect the changes in energy balance caused by reproductive rhythm and weaning management.

Table 3.3 Coefficients of correlation between body condition and energetic mobilization measurements.

		Mating			Delivery				10d after delivery			
		PFT	$NEFA_b$	$NEFA_r$	BW	PFT	$NEFA_b$	$NEFA_r$	BW	PFT	$NEFA_b$	$NEFA_r$
Mating	BW	0.53*	0.10	0.02	0.64*	0.41*	0.32*	0.23*	0.57*	0.45*	0.05	0.14
	PFT		0.18	0.25*	0.35*	0.31*	0.24*	0.12	0.34*	0.29*	0.24*	0.26*
	$NEFA_b$			0.65*	−0.09	−0.09	0.16	0.09	−0.03	−0.03	0.09	0.12
	$NEFA_r$				−0.07	−0.09	0.18	0.14	−0.08	−0.06	0.18	0.13
Delivery	BW					0.64*	0.10	0.07	0.83*	0.62*	− 0.01	0.11
	PFT						0.02	0.00	0.55*	0.51*	0.03	0.04
	$NEFA_b$							0.72*	0.08	0.04	0.14	0.11
	$NEFA_r$								−0.01	0.04	0.14	0.20*
10 d after deliverv	BW									0.67*	− 0.13	−0.06
	PFT										0.09	0.20*
	$NEFA_b$											0.69*

* P-value<0.05. Body weight (BW), perirenal fat thickness (PFT), basal non-esterified fatty acids concentration ($NEFA_b$), and non-esterified fatty acids after lipolysis stimulation ($NEFA_r$).

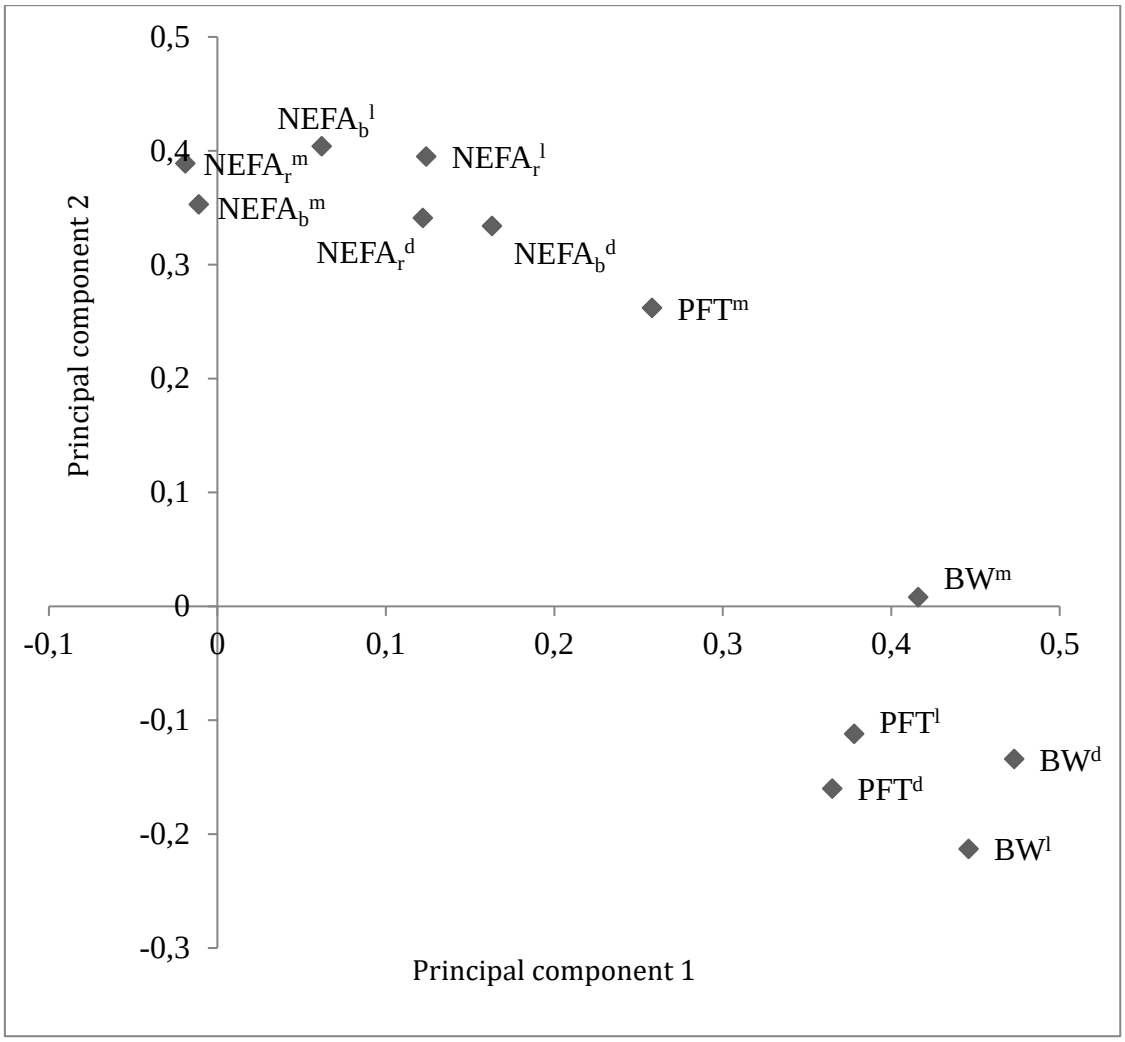

Figure 3.1 Projection of the traits in the plane defined by the two first principal components. BW: Body weight, PFT: perirenal fat thickness, $NEFA_b$: basal non-esterified fatty acids concentration, $NEFA_r$: non-esterified fatty acids after lipolysis stimulation. Superscripts m, d and l mean variable measured at mating, delivery and 10 d after delivery, respectively.

We conclude that although body weight and perirenal fat thickness are good predictors of body condition, NEFA measurements should be used when an accurate measurement of energetic mobilization is needed. We also conclude that measuring NEFA after stimulating lipolysis with an adrenergic agent is going to give similar results as basal NEFA, thus it does not seem to be required for a prediction of does' fat reserves mobilization, unless the experiment requires a more accurate prediction of the additional capacity of the does for energetic mobilization.

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Chapter 4

CORRELATED RESPONSE IN BODY CONDITION AND FAT MOBILIZATION IN RABBITS SELECTED FOR LITTER SIZE VARIABILITY

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ABSTRACT

The aim of this study was to evaluate the correlated response in body condition and fat reserves mobilization in two rabbit lines divergently selected by litter size variability during seven generations. Variability of litter size was estimated as phenotypic variance of litter size within female after correcting for the year-season and lactation status effects. A total of 80 females from the high line and 74 females from the low line were used in this study. Body condition was measured as perirenal fat thickness. Mobilization of fat reserves was measured as the increment in non-esterified fatty acids levels from basal concentration until adrenergic stimulation by isoproterenol ($NEFA_r$) at second mating, delivery and 10 d after delivery. Data were analysed using Bayesian methodology. For perirenal fat thickness, the line selected for increasing litter size variability showed lower fat thickness than the homogenous line at delivery (-0.16 mm, P = 0.86), and this difference remained at 10 d after delivery (-0.17 mm, P = 0.86). The homogenous line exhibited 30% more concentration in $NEFA_r$ (P = 0.96) at delivery than the heterogeneous one. In conclusion, a decrease in litter size variability showed a favourable effect on body condition and fat reserve mobilization. In this regard, the more homogenous line for litter size seems to adapt better to adverse environments, as it has a greater capacity to mobilize energy reserves at delivery than the heterogeneous line. Females from the line selected for litter size homogeneity are, therefore, more resilient than those of the heterogeneous line.

Keywords: Body condition, litter size variability, non-esterified fatty acids, perirenal fat thickness, resilience.

INTRODUCTION

In prolific species such as rabbits and pigs, variability in litter size within a female is linked to her capacity to cope with environmental changes. This adaptation often involves changes in mobilization of body energy reserves, and consequently on animal body condition (Rauw, 2009). There is evidence to support that this

environmental sensitivity may be under genetic control (Mulder *et al.*, 2013), whereby genes controlling environmental sensitivity can also control body condition. A divergent selection experiment for litter size environmental sensitivity has been carried out successfully in rabbits. After seven generations of selection, the low litter size variability line was 30% more homogeneous in litter size than the high line (Argente *et al.,* 2014a). Our hypothesis is that the more uniform line is also more resilient when facing changes in microenvironment. We know, for example that stress has a negative effect on resource allocation and body condition (Elsasser *et al.,* 2000; Broom, 2008). Selection for litter size environmental sensitivity may modify body condition. Body condition is related to body fat reserves (review by Chilliard, 1993), whose mobilization can be measured throught non-esterified fatty acids (NEFA) levels in blood (Belstra *et al.,* 1998 in pigs; Chilliard *et al.,* 1998; Fortun-Lamothe, 2006 in rabbits). It would be interesting to examine how selection for environmental sensitivity affects the mobilization of fat reserves.

The objective of this study was to analyse the correlated response to selection for litter size variability in body condition and fat reserves mobilization in rabbit females.

MATERIALS AND METHODS

Animals

Animals came from the seventh generation of a divergent selection experiment for litter size variability. Data from 80 females from the high line and 74 females from the low line were used in this study. All females were primiparous. Variability of litter size was estimated as phenotypic variance of litter size within female after correcting for the effects of year-season and lactation status (see more details in Argente *et al*., 2014a). All animals were kept on the farm at the Miguel Hernández University of Elche (Spain). Rabbits were fed a standard commercial diet (218 g acid detergent fibre and 174 g crude protein per kg of dry matter; Cunilactal, Nutreco). Food and water were provided ad libitum. Females were housed in individual cages

under a constant photoperiod of 16 h continuous light: 8 h continuous darkness and controlled ventilation throughout the experiment. They were first mated at 18 wk of age and at 10 d after parturition thereafter. Litters were not standardized.

All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee (Reference number DTA-MJA-001-11), according to Council Directives 98/58/EC and 2010/63/EU.

Traits

Body fat reserves and mobilization of adipose tissue were recorded at three different physiology stages of the doe; second mating, delivery and 10 d after delivery. Body fat reserves were measured as perirenal fat thickness by ultrasound imaging as described by Pascual *et al*. (2004), using Justvision 200 SSA-320A Toshiba ultrasound equipment. Mobilization of fat reserves was measured as basal non-esterified fatty acids ($NEFA_b$) and increase of blood NEFA after injection of isoproterenol ($NEFA_r$), an adrenergic agent which increases lipolysis. $NEFA_r$ is also known as the lipolytic potential of fat reserves (Theilgaard *et al*., 2005). Blood was sampled before and 7.5 min after injection of 50 µg of isoproterenol per kg of body weight (Sigma 15627). This time interval and concentration of isoproterenol were established as appropriate by Theilgaard *et al*. (2005) for assessing the lipolytic potential in rabbits. Blood samples were obtained from the central ear artery early in the morning, before feed was distributed, in order to prevent the effect of feeding, as proposed by Theilgaard *et al*. (2005). The samples were centrifuged immediately after sampling (4,000 r.p.m., 4 ºC, 15 min) and plasma was stored at -20ºC until further analysis. Plasma NEFA concentrations were determined using the *in vitro* enzymatic colorimetric methodology prepared by the NEFA test Wako C (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Samples were analysed with a UV spectrophotometer (Hewlett Packard Model 8453).

Statistical Analysis

Differences between lines were estimated using a model with effects of line-physiological status, season, lactation status at mating and permanent effect of the doe.

All analyses were performed using Bayesian methodology. Bounded uniform priors were used for all effects with the exception of the doe permanent effect, considered independently normally distributed with mean $\mathbf{0}$ and variance $\mathbf{I}\sigma^2_p$. Residuals were a priori independently normally distributed with mean $\mathbf{0}$ and variance $\mathbf{I}\sigma^2_e$. The priors for the variances were also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for all procedures. After some exploratory analyses, we used a chain of 60,000 samples, with a burn-in period of 10,000. Only one of every 10 samples was saved for inferences. Convergence was tested using the Z criterion of Geweke (Sorensen and Gianola, 2002) and Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992).

RESULTS AND DISCUSSION

Animal breeding has traditionally been focused on increasing productivity. However, success in animal improvement appears to be seriously compromised by animal health and welfare (Rauw *et al.,* 1998). Enhancing resilience in animals would be an interesting breeding goal for livestock, as animals may express their high production potential while being less affected by environmental conditions (Knap, 2005). We have proposed that resilience in dams is related to the variation in litter size throughout their reproductive lifespan (Argente *et al.,* 2014b). There is no available information on whether selection for litter size environmental sensitivity can affect body condition. Table 4.1 shows means and coefficients of variation for body condition and fat mobilization reserves at mating, delivery and 10 d after delivery in the high and low lines. The basal NEFA concentration and lipolytic potential of fat reserves were highly variable traits, exhibiting larger coefficients of variation than body weight and fat reserves.

Table 4.1 Mean and coefficient of variation (CV) for body condition and fat mobilization reserves at second mating, delivery and 10 d of lactation in the high and low lines.

		High line (n=80)		Low line (n=74)	
		Mean	CV	Mean	CV
Perirenal fat thickness (mm)	Mating	9.33	0.09	9.34	0.09
	Delivery	9.00	0.10	9.19	0.10
	Lactation at 10 d	9.07	0.10	9.26	0.11
$NEFA_b$ (mmol/l)	Mating	0.53	0.47	0.53	0.49
	Delivery	0.68	0.44	0.63	0.51
	Lactation at 10 d	0.53	0.41	0.54	0.39
$NEFA_r$ (mmol/l)	Mating	0.41	0.76	0.28	0.96
	Delivery	0.32	0.72	0.42	0.64
	Lactation at 10 d	0.30	0.77	0.27	0.85

$NEFA_b$: basal non-esterified fatty acid levels before adrenergic stimulation. $NEFA_r$: response in non-esterified fatty acid levels from basal concentration until adrenergic stimulation.

Features of the estimated marginal posterior distributions of the differences between the high and low lines for all traits are displayed in table 4.2. Marginal posterior distributions were approximately normal, so the mode, mean and median were similar. All Monte Carlo standard errors were very small and lack of convergences was not detected by the Geweke test. When $|D_{H-L}| > 0$, we consider that there is enough evidence about the high and low lines are different, if the probability of $|D_{H-L}|$ is more than 0.80. After seven generations of selection for litter size variability, there is some evidence that the high line had lower body fat than the low line at delivery (-0.16 mm, P = 0.86) and 10 d after delivery (-0.17 mm, P = 0.86), thus the line selected for homogeneity had a better body condition.

Table 4.2 Features of the estimated marginal posterior distribution of the differences between the high and low lines for body condition measurements at mating, delivery and 10 d after delivery.

		D_{H-L}	$HPD_{95\%}$	P
Perirenal fat thickness (mm)	Mating	0.02	-0.25, 0.33	0.63
	Delivery	-0.16	-0.44, 0.13	0.86
	10 d after Delivery	-0.17	-0.47, 0.12	0.86
$NEFA_b$ (mmol/l)	Mating	-0.02	-0.11, 0.09	0.63
	Delivery	0.04	-0.07, 0.14	0.76
	10 d after Delivery	-0.02	-0.12, 0.09	0.65
$NEFA_r$ (mmol/l)	Mating	0.13	0.03, 0.23	0.99
	Delivery	-0.09	-0.19, -0.01	0.96
	10 d after Delivery	-0.02	-0.12, 0.09	0.65

D_{H-L}: median of the difference between the high and low lines. $HPD_{95\%}$: highest posterior density region at 95%. P: probability of the difference being >0 when D_{H-L} >0 and probability of the difference being < 0 when D_{H-L}<0. $NEFA_b$: basal non-esterified fatty acids levels before adrenergic stimulation. $NEFA_r$: response in non-esterified fatty acid levels from basal concentration until adrenergic stimulation.

The concentration of NEFA in blood is a useful biochemical marker for quantifying fat mobilization from body reserves in several species (Belstra *et al.,* 1998 in pigs; Chilliard *et al.,* 1998; Fortun-Lamothe, 2006 in rabbits). No relevant differences were found for basal NEFA levels between lines at mating, delivery and 10 d after delivery. Blood NEFA level in response to the adrenergic agent isoproterenol has proved to be useful to evaluate the lipolytic potential of fat reserves *in vivo* (Chilliard *et al.*, 1998). At delivery, a time of high energy demands, the low line exhibited a 30% higher concentration in $NEFA_r$ (P = 0.96) than the high line, thus the homogenous line had a better mobilization of fat reserves. Therefore, the does from

the low line exhibited better body condition and more efficient management in their body reserves under strong energetic demands.

Delivery and lactation are stressful stages for female mammals (Hydbring *et al.,* 1999; Gellrich *et al.,* 2015). Several studies have reported that stress negatively affects the immune system, and therefore disease susceptibility (see review by Webster-Marketon and Glaser, 2008). Stress also has a negative effect on resource allocation and body condition (Elsasser *et al.,* 2000; Broom, 2008). Because of this, body condition has been proposed as an indicator for animal health and welfare (Blache *et al.,* 2011). We found a better body condition and higher fat mobilization in the line selected for homogeneity than in the heterogeneous line, which suggests a higher animal health and welfare in this line. Greater efficiency in mobilization of fat reserves in the low line does may enable them to adapt better to environmental changes.

CONCLUSION

Selection for decreasing variability in litter size seems to improved female body condition and fat mobilization, which is related to a higher degree of health and welfare in the animal. The does selected for litter size homogeneity could be able to better deal with situations of high energy demand than does with higher litter size variability, being more resilient.

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Chapter 5

CORRELATED RESPONSE IN LITTER SIZE COMPONENTS IN RABBITS SELECTED FOR LITTER SIZE VARIABILITY

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ABSTRACT

A divergent selection experiment for litter size environmental variability has been carried out in rabbits at the University Miguel Hernández of Elche in Spain over seven generations. Environmental variability of litter size was estimated as phenotypic variance within female after correcting for year-season and lactation status. The aim of this study was to analyse the correlated responses to selection in litter size and litter size components. A total of 94 females from the high line and 82 females from the low line were used in this study. Ovulation rate (OR) and number of implanted embryos (IE) were measured by laparoscopy at 12 d of the second gestation. The total number of kits born (TNB) and alive (NBA) were also recorded at second parity. Embryonic (ES), fetal (FS) and prenatal (PS) survival were estimated as IE/OR, TNB/IE and TNB/OR, respectively. Data were analysed using Bayesian methodology. After seven generations of selection, ovulation rate was similar in both lines. The line selected for homogeneity in litter size showed more embryos at implantation (11.53 embryos vs 10.20 embryos, P = 1.00) and higher embryonic survival than the heterogeneous line (0.87 vs. 0.78, P = 1.00). A higher uterine overcrowding of embryos in the homogeneous line did not penalise fetal survival, and as a result, this line continued showing a greater number of kits born at birth (+0.98 kits, P = 0.96). In conclusion, a decrease in litter size variability showed a favourable effect on embryonic survival leading to a higher litter size at birth.

Keywords: implanted embryos, litter size, ovulation rate, rabbit, residual variance.

INTRODUCTION

Interest in the genetic determination of environmental variance is increasing, as the livestock industry is demanding a more homogeneous production (Mulder *et al.* 2008); for example, increasing uniformity in litters can help management and increase litter viability. On the other hand, a decrease in environmental variance will increase the heritability (Formoso-Rafferty et al., 2017), being particularly

interesting for increasing the response to selection in low heritability traits, such as litter size. A direct divergent selection experiment for litter size environmental variance is currently being carried out in rabbits. The experiment has had some success, showing a difference of 30% between the divergent lines (Argente *et al.* 2014a). Litter size environmental variance is related to litter size, but the sign of this relationship is controversial. In a closely related trait in rabbits, uterine capacity, Ibáñez-Escriche *et al*. (2008) found a negative relationship between the environmental variance and the mean of the trait, but reanalysing the data after normalising the residuals, Yang *et al.* (2011) found a low positive relationship between both traits. In pigs' litter size, Yang *et al.* (2011) found that after the transformation the relationship between litter size and litter size environmental variance changed from -0.6 to +0.7. Hence, it will be interesting to learn how this selection process is affecting litter size, and also at which gestation moment the selection process is acting. The objective of this study is to analyse the correlated responses to selection for litter size environmental variability on litter size components.

MATERIALS AND METHODS

Animals

Animals came from two divergent rabbit lines selected for residual variance of litter size over seven generations. A total of 94 females from the high line and 82 females from the low line were used in this study. Selection was based on phenotypic variance of litter size within female after correcting litter size for year-season and lactation status. As all litters have almost the same genetic determination (Piles *et al.* 2006) and the same environmental permanent effects, after correcting for systematic effects, the phenotypic variance intra-doe is a record of its residual variance (see more details in Argente *et al.* 2014a). All animals were kept on a farm at the Miguel Hernández University of Elche (Spain). Rabbits were fed a standard commercial diet (218 g acid detergent fibre and 174 g crude protein per kg of dry matter; Cunilactal, Nutreco). Food and water were provided *ad libitum*. Females were kept in individual cages under a constant photoperiod of 16 h continuous light:

8 h continuous darkness and controlled ventilation. They were first mated at 18 wk of age and at 10 d after parturition thereafter. Litters were not standardised. A laparoscopy was performed in all females at 12 d of second pregnancy, in order to estimate ovulation rate and number of implanted embryos. The laparoscopy technique is described in detail by Argente *et al.* (2003), and previously Santacreu et al (1990) showed that litter size is not affected by the performance of this technique.

All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee (Reference number DTA-MJA-001-11), in accordance with Council Directives 98/58/EC and 2010/63/EU.

Traits

The analysed traits were ovulation rate (OR), number of implanted embryos (IE), total number of kits born (TNB) and alive (NBA) at second parity, embryonic survival (ES = IE / OR), fetal survival (FS = TNB / IE), and prenatal survival (SP = TNB / OR).

Statistical Analyses

Lines were compared using a model including effects of line, season and lactation status at mating (lactating or non-lactating). Correlation coefficients between the residuals of the traits from a model including the effects of season and lactation status were estimated in each line separately. All analyses were performed using Bayesian methodology. Bounded uniform priors were used for all effects. Residuals were a priori normally distributed with mean $\mathbf{0}$ and variance $\mathbf{I}\sigma^2_e$. The prior for the variance was also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for all procedures. We used a chain of 60,000 samples, with a burn-in period of 10,000. Only one out of every 10 samples was saved for inferences. Convergence was tested using the Z criterion of Geweke (Sorensen and Gianola 2002) and Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992).

RESULTS AND DISCUSSION

Homogeneity is an economically important trait in livestock production (Mulder *et al.* 2008). We previously carried out a divergent selection experiment on litter size variability in rabbits successfully (Argente *et al.* 2014a). In this paper, we examine the consequences that this selection process had on litter size components. Table 5.1 shows the means and coefficients of variation for litter size components in the high and low lines. The homogenous line for litter size showed lower variability (CV) for all studied traits.

Table 5.1 Mean and coefficient of variation (CV) for the traits in the high and low lines.

	High line		Low line	
	Mean	CV	Mean	CV
OR, ova	13.34	0.19	13.36	0.17
IE, embryos	10.20	0.32	11.53	0.24
ES, embryos / ova	0.78	0.29	0.87	0.19
FS, kits / embryos	0.69	0.36	0.69	0.35
PS, kits / ova	0.54	0.47	0.59	0.40
TNB, kits	7.16	0.52	7.94	0.44
NBA, kits	6.03	0.67	6.23	0.67

OR: ovulation rate. IE: number of implanted embryos. ES: embryonic survival. FS: fetal survival. PS: prenatal survival. TNB: total number of kits born. NBA: number of kits born alive.

Table 5.2 presents correlated response to selection. Marginal posterior distributions of the differences between lines were approximately normal, thus mode, mean and median were similar. All Monte Carlo standard errors were very small and lack of convergences was not detected by the Geweke test. When $|D_{H-L}| > 0$, we consider that there is enough evidence about the high and low lines are different, if the probability of $|D_{H-L}|$ is more than 0.80. After seven generations of selection, ovulation rate was similar in both lines, showing a mean value in agreement with the range reported in rabbit literature (Blasco *et al.* 1993; García and Baselga 2002; Laborda *et al.* 2011).

For embryonic survival, the heterogeneous line had lower values (78%) than those reported in the literature, which vary from 86% to 90% (Adams 1960; Blasco *et al.* 1993; García and Baselga 2002; Laborda *et al.* 2012; Ziadi *et al.* 2013). The homogeneous line showed more embryos at implantation (P =1.00) and a higher embryonic survival than the heterogeneous line (0.87 vs. 0.78, P = 1.00), but similar fetal survival, leading to a greater number of kits at birth than the heterogeneous line (P = 0.96). Therefore, selection for residual litter size variability has a negative correlated response with number of implanted embryos and with litter size. This is in agreement with Ibáñez-Escriche *et al.* (2008), who reported a negative correlation between uterine capacity and its residual variability in rabbits, a trait highly correlated with litter size (Argente *et al.* 2000). Our results show that the difference in litter size between lines was established at implantation. There is evidence that maternal stress around the time of implantation increases the failure rate in blastocyst implantation (Burkuš *et al.* 2015). We hypothesise that the line selected for heterogeneity in litter size should be more sensitive to stress and diseases than the homogeneous line. In this regard, Argente *et al.* (2014b) found a lower immune response to pathogenic agents in females from the heterogeneous line, showing greater sensitivity to diseases.

Table 5.2 Correlated response. Features of the estimated marginal posterior distribution of the differences between the high and low lines.

	D_{H-L}	$HPD_{95\%}$	P
OR, ova	-0.15	-0.89, 0.61	0.65
IE, embryos	-1.48	-2.50, -0.56	1.00
ES, embryos / ova	-0.09	-0.15, -0.03	1.00
FS, kits / embryos	-0.01	-0.09, 0.06	0.57
PS, kits / ova	-0.06	-0.14, 0.01	0.94
TNB, kits	-0.98	-2.10, 0.15	0.96
NBA, kits	-0.35	-1.61, 0.85	0.71

D_{H-L}: mean of the difference between the high and low lines. $HPD_{95\%}$: highest posterior density region at 95%. P: probability of the difference being >0 when D_{H-L} >0 and probability of the difference being < 0 when D_{H-L}<0. OR: ovulation rate. IE: number of implanted

embryos. ES: embryonic survival. FS: fetal survival. PS: prenatal survival. TNB: total number of kits born. NBA: number of kits born alive.

Features of the estimated marginal posterior distributions of the phenotypic correlations between traits are summarised in Table 5.3. We considered a phenotypic correlation irrelevant when it was less than 0.1 in absolute value. In a frequentist context we would offer the result of a signification test for the correlation coefficient, which has the drawback of being dependent on sample size and being rather uninformative, as 'n.s.' does not mean a null correlation. Here we offer the actual probability P_R of a correlation being relevant, or at least non-irrelevant. The phenotypic correlation between ovulation rate and number of implanted embryos was positive (P = 1.00), and relevant in both lines (P_R was 0.98 in the high line and 1.00 in the low line). However, the correlation was near twice as high in the homogenous line as in the heterogeneous line. Ovulation rate showed a negative correlation with embryo survival (P = 1.00), but was only relevant in the heterogeneous line (P_R= 0.93). Both results would be in agreement with the lower number of implanted embryos in this line (Table 5.2). The other correlations were more similar between lines, confirming that the differences appear at early stages of gestation.

Table 5.3 Phenotypic correlation between traits in the high and low lines. Features of the posterior distributions.

	High line				Low Line			
Trait	Mean	$HPD_{95\%}$	P	P_R	Mean	$HPD_{95\%}$	P	P_R
OR, IE	0.30	0.12 , 0.49	1.00	0.98	0.59	0.44 , 0.74	1.00	1.00
OR, ES	-0.29	-0.48 , -0.09	1.00	0.97	-0.15	-0.39 , 0.07	0.90	0.70
OR, FS	0.07	-0.13 , 0.28	0.74	0.44	0.08	-0.15 , 0.31	0.75	0.50
OR, PS	-0.11	-0.30 , 0.13	0.83	0.52	-0.01	-0.23 , 0.23	0.51	0.41
EI, ES	0.81	0.73 , 0.88	1.00	1.00	0.69	0.56 , 0.81	1.00	1.00
EI, FS	-0.04	-0.25 , 0.18	0.65	0.39	-0.10	-0.34 , 0.13	0.79	0.55
EI, PS	0.52	0.36 , 0.67	1.00	1.00	0.26	0.06 , 0.48	0.99	0.93
ES, FS	-0.10	-0.30, 0.12	0.83	0.55	-0.19	-0.43 , 0.02	0.95	0.79
ES, PS	0.57	0.43 , 0.71	1.00	1.00	0.34	0.12 , 0.54	1.00	0.98
FS, PS	0.72	0.62 , 0.82	1.00	1.00	0.85	0.78 , 0.91	1.00	1.00
OR, TNB	0.29	0.10 , 0.48	1.00	0.97	0.43	0.23 , 0.62	1.00	1.00
IE, TNB	0.67	0.55, 0.78	1.00	1.00	0.50	0.32 , 0.67	1.00	1.00
ES, TNB	0.48	0.31 , 0.63	1.00	1.00	0.23	-0.01 , 0.44	0.98	0.87
FS, TNB	0.67	0.57 , 0.79	1.00	1.00	0.80	0.67 , 0.92	1.00	1.00
PS, TNB	0.91	0.87 , 0.94	1.00	1.00	0.89	0.85 , 0.94	1.00	1.00

HPD95% = high posterior density interval at 95%. P: probability of the phenotypic correlation coefficient being greater than zero when positive, or lower than zero when negative. P_R: probability of relevance; i.e., probability of the correlation coefficient higher than 0.1 in absolute value. OR: ovulation rate. ES: embryonic survival. FS: fetal survival. PS: prenatal survival. IE: number of implanted embryos. TNB: total number of kits born.

In conclusion, selection for litter size variability showed a negative correlated response in embryonic survival, which continued at birth for litter size.

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Chapter 6

CORRELATED RESPONSE IN EARLY EMBRYONIC DEVELOPMENT IN RABBITS SELECTED FOR LITTER SIZE VARIABILITY

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Abstract

A divergent selection experiment for litter size variability was carried out in rabbits. Variability of litter size was estimated as phenotypic variance of litter size within female. The aim of this study was to assess the effect of selection for litter size variability on early embryonic development and survival after seven generations of selection. A total of 30 non-lactating multiparous does per line were used in this study. Ovulation rate and early embryonic development were analysed using Bayesian methodology. Ovulation rate was not affected by the selection process. At 28 h of gestation, embryonic development and survival were similar in both lines. At 48 h of gestation, the majority of embryos were catalogued as early morulae in the high litter size variability line (79.54%). This line had a 27% more percentage of early morulae (P=0.94) and a 26% lower percentage of compacted morulae (P=0.93%) than the low line. At 72 of gestation, the high line had 1.59 embryos less than the more homogeneous line (P=0.85), as a consequence of its lower embryonic survival (0.60 vs 0.74, P=0.93). The line selected for increasing litter size variability continued to show a higher percentage of early morulae (21.01% vs 3.69%, P=0.93) and lower percentage of compacted morulae and blastocyst (78.99% vs 96.31%, P=0.94) than homogenous line, i.e. the high line also had a lower embryonic development at 72 h of gestation. In conclusion, selection for homogeneity in litter size evidenced a positive impact on embryonic traits.

Keywords: Blastocysts, embryonic survival, morulae, ovulation rate, residual variance.

Introduction

Environmental sensitivity in animals has a considerable impact on their productivity (Rauw and Gomez-Raya, 2015). Selection for reducing environmental variance can lead to animals performing well in adverse environments (Mulder *et al.,* 2013). A divergent selection experiment for litter size variability has been carried out successfully in rabbits; after seven generation of selection, the line selected to increase litter size variability showed a greater variability (+1.19 kits^2) and a lower mean in litter size (-0.70 kits) than the low litter size variability line, as

consequence of a lower number of implanted embryos (-1.38 embryos) (Argente *et al.,* 2014a). In addition, this line had a less resilience, i.e. greater sensitivity to illness and stressful conditions (García *et al.,* 2012; Argente *et al.,* 2014b). Stress in dams increases the failure rates in blastocyst implantation (Liu *et al.,* 2015; Burkuš *et al.,* 2015) through changes in expression patterns of genes involved in embryo development (Marco-Jiménez *et al.,* 2013; Silva *et al.,* 2013). Asynchrony between embryonic development and oviductal functionality plays an important role in early embryonic losses (Geisert and Schmitt, 2002). Our working hypothesis is that lower implantation rate in the line selected to increase litter size variability can be related to a retarded embryonic development in relation to the oviductal functionality.

The aim of this study was to assess the effect of selection for litter size variability on early embryonic development and survival in rabbits.

Materials and Methods

All experimental procedures involving animals were approved by the Research Ethics Committee of Miguel Hernández University, Elche on 21 June 2011 (Reference 98 number DTA-MJA-001-11), in accordance with Council Directives 98/58/EC and 2010/6/EU.

Animals

Animals came from the seventh generation of a divergent selection experiment for litter size variability, measured as phenotypic variance of litter size within does after correcting for the effects of year-season and parity-lactation status (first parity, and lactating or not at mating in other parities). Details of the experiment can be found in Argente *et al*. (2014a). All animals were bred at the farm of the Miguel Hernández University, Elche. They were kept under a constant photoperiod of 16 h continuous lighting: 8 h continuous darkness and controlled ventilation.

Traits

A total of 30 non-lactating multiparous does per line were used in this experiment. Does were euthanized at 28, 48 or 72 h post-mating by intravenous administration

of sodium thiopental in a dose of 50 mg/kg of body weight (Thiobarbital, B. Braun Medical S.A., Barcelona, Spain). The entire reproductive tract was immediately removed. Ovulation rate (OR) was estimated as the number of corpora haemorrhagica. The number of normal embryos (NE), abnormal embryos, and oocytes were counted after collection by perfusion of each oviduct and uterine horns with 10 mL of Dulbecco's phosphate buffered saline containing 0.2% of bovine serum albumin. Embryos were classified as normal when they presented homogeneous cellular mass and intact zona pellucida and mucin coat (Maurer, 1978), using a binocular stereoscopy microscope (Leica Mz 9.5-600x). At 28 h of gestation, normal embryos were classified as 2-cell embryos (2-cells) or 4-cell embryos (4-cells). At 48 h of gestation, normal embryos were classified as early morulae (EM) or compacted morulae (CM). At 72 h of gestation, normal embryos were classified as early morulae, compacted morulae or blastocysts (B). In all cases, number of 2-cells, 4-cells, early morulae, compact morulae and blastocysts were expressed as a percentage from the number of normal embryos. Early embryonic survival (EES) was estimated as normal embryos divided by ovulation rate.

Statistical analyses

All traits were analysed with a model including the fixed effects of line and season. The model for OR also included pregnancy stage (28 h, 48 h and 72 h post-mating) as fixed effect. The traits were analysed using Bayesian methodology. Bounded flat priors were used for all unknowns. Residuals were independently normally distributed with mean $\mathbf{0}$ and variance $\mathbf{I}\sigma_e^2$. The priors for the variances were also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for performing the analyses of differences between lines (http://www.dcam.upv.es/dcia/ablasco/Programas/THE_PROGRAM_Rabbit.pdf). After some exploratory analyses, we used a chain of 60,000 samples, with a burn-in period of 10,000 and only one of every 10 samples saved for inferences. Convergence was tested using the Z criterion of Geweke (Sorensen and Gianola, 2002) and Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992). In all Bayesian analysis, Monte Carlo

standard errors were small and lack of convergence was not detected by the Geweke test. An advantage of the Bayesian approach through MCMC procedures is the ease of computation of confidence intervals and probabilities (see reviews by Blasco, 2001 and 2005). Bayesian statistics gives a new approach to the description of the uncertainty against classical statistics. For example, we can give the median in each line and the precision of our estimation, finding the shortest interval with 95% probability of containing the true value (what is called the highest posterior density interval at 95%). Note that this interval is not dependent on the estimate we give, and it can be asymmetric about the median. Besides, we are interested in estimating differences between the high and low lines (D_{H-L}), thus we may also calculate the probability of this difference being greater than zero [$P(D_{H-L}>0)$].

Results and discussion

Table 6.1 shows the features of the estimated marginal posterior distributions of the differences between lines (D_{H-L}) for ovulation rate and early embryonic development. When $|D_{H-L}| > 0$, we consider that there is enough evidence about the high and low lines are different, if the probability of $|D_{H-L}|$ is more than 0.80 (P in Table 6.1). Do not confuse P with P-value (Blasco 2001, 2005). Johnson (2013) has shown that the evidence provided by P-values is lower than they indicate. For example, a P-value of 0.05 only gives 67% to 75% of evidence, i.e. around a 25% of false positives appear with P-values of 0.05. Then P is the actual probability, thus we chose 80% as evidence enough. According to the value of P, we see that both lines showed a similar ovulation rate (P = 0.70).

Table 6.1. Features of the estimated marginal posterior distribution of the differences between the high and low lines selected for litter size variability.

	High line[a]	Low line[a]	D_{H-L}	$HPD_{95\%}$	P
OR[b]	12.43 (2.30)	12.08 (2.35)	0.35	-1.01, 1.57	0.70
28 h post-mating[c]					
NE	9.95 (2.86)	10.24 (2.37)	-0.29	-3.44, 3.06	0.55
2C, %	42.64 (29.35)	52.86 (32.21)	-10.18	-41.85, 23.10	0.75
4C, %	57.36 (33.54)	47.14 (31.34)	10.81	-21.43, 40.45	0.75
EES	0.81 (0.10)	0.84 (0.12)	-0.05	-0.25, 0.18	0.63
48 h post-mating[c]					
NE	9.92 (2.92)	10.20 (2.35)	-0.27	-3.65, 2.85	0.58
EM, %	79.54 (38.68)	53.43 (37.52)	26.81	-6.06, 62.12	0.94
CM, %	20.46 (37.40)	46.57 (38.67)	-26.16	-60.50, 8.28	0.93
EES	0.79 (0.11)	0.85 (0.12)	-0.06	-0.28, 0.14	0.72
72 h post-mating[c]					
NE	7.56 (2.12)	9.18 (3.01)	-1.59	-4.76, -1.42	0.85
EM, %	21.01 (25.71)	3.69 (20.19)	17.36	-6.42, 39.86	0.93
CM, %	27.38 (34.65)	33.63 (37.19)	-7.17	-42.28, 26.07	0.67
B, %	51.61 (44.43)	62.68 (44.67)	-11.27	-50.74, 31.70	0.71
EES	0.60 (0.10)	0.74 (0.11)	-0.14	-0.34, 0.05	0.93

a: mean (standard deviation). b: 30 does per line. c: 10 does per line. D_{H-L}: median of difference between the high and low lines. $HPD_{95\%}$: highest posterior density region at 95%. P: probability of the difference being >0 when D_{H-L} >0 and probability of the difference being < 0 when D_{H-L}<0. OR: ovulation rate. NE: Number of normal embryos. 2C: 2-cell embryos. 4C: 4-cell embryos. EM: early morulae. CM: compacted morulae. B: blastocysts. 2C, 4C, EM, CM and B were expressed as a percentage of their respective number of normal of embryos (NE). EES: early embryonic survival (NE/OR).

At 28 h of gestation, the high and low lines exhibited similar numbers of recovered embryos (P = 0.55) and embryonic survival rate (P = 0.63). No difference in embryonic development was found between lines (P = 0.75). We observed similar percentage of embryos in 2-cell stage than in 4-cell stage (about a 50% in each one) in both line. The literature indicates that the majority of embryos are in 2-cell stage at 25 h post-mating (Peiró *et al.,* 2007) and in 4-cell stage at 30 h post-mating (Peiró *et al.,* 2015). Our results are in agreement with these studies, corroborating that embryonic development at 28 h post-mating is an intermediate stage between 25 h and 30 h post-mating.

At 48 h of gestation, no difference was found in number of recovered embryos (P = 0.58) and embryonic survival (P = 0.72) between the high and low lines. The majority of embryos were catalogued as early morulae in the high line (79.54%). The high line showed a 27% (2.8 embryos) higher percentage of early morulae (P=0.94) and a 26% lower percentage of compacted morulae than the low line (20.46% vs 46.57%, respectively, P=0.93). Hence, increasing litter size variability revealed a negative effect on early embryonic development.

At 72 h of gestation, the difference for number of normal embryos increased, and the high line had 1.59 embryos less than the more homogeneous line (P = 0.85). Embryonic survival was also lower in the high line than in the low line (0.60 vs 0.74, P=0.93). The line selected for increasing litter size variability had higher percentage of early morulae (21.01% in the high line vs 3.69% in the low line, P = 0.93) and a lower percentage of compacted morulae and blastocysts (78.99% in the high line vs 96.31% in the low line, P=0.94). Therefore, the high line continued to show a lesser embryonic development than the homogeneous line at 72 h of gestation. The embryonic development in this line was also smaller than that reported in others maternal lines (Mocé *et al,* 2004; Peiró *et al.,* 2007; Argente *et al.,* 2010), which exhibited a minor percentage of early morulae (10% - 14%) and a major percentage of compacted morulae and blastocyst (85% - 95%). Several studies have found that embryos with a lower development rate showed a higher mortality rate than those with a more advance development during gestation (Torres *et al.,* 1987) and at birth Murakami and Imai (1996). The high line had a lower number of implanted embryos than the

low line after seven generations of selection (10.18 embryos in the high line vs 11.49 embryos in the low line, Argente *et al.,* 2014a). This difference was maintained at birth for litter size (Blasco *et al.,* 2017). These results would be in agreement with lesser embryonic development at 48 and 72 h of gestation in the high line, which posteriorly has a negative effect on embryonic survival.

In previous studies, the high line showed a higher subclinical immune response, which is related to a higher sensitivity to usual microenvironmental microorganisms in the farm (García *et al.,* 2012; Argente *et al.,* 2014b). These results are in agreement with a higher sensitivity to stress and a larger probability to ill in the high line than the low one. We hypothesize that the line selected for increase litter size variability can delay the development of the embryos, as a consequence of higher sensitivity to illness and to stress than the homogenous line. It is known that embryo development can be delayed under stress due to disruption of protein involving embryonic growth (review by Puscheck *et al.,* 2015). For example, lack of DICER1, MATER, ZAR1, PADI6, and SEBOX does not allow embryo to develop beyond the 2-cell stage, while embryo is unable to reach the 8-cell or morulae stage in absence either of SMARCA4, DNMT1, DNMT3A, TET, KLF4 or OCT4, NANOG, SOX2, respectively (see review by Argente, 2016). Moreover, the survival of an embryo that reaches the oviduct environment in a less developed state than the oviduct per se will be compromised in the early stages of pregnancy due to an asynchrony problem (review by Geisert and Schmitt, 2002). It has reported that, although, lesser development embryos can survive beyond implantation, they would probably die soon after that due to fetal competence for uterine space, and a poor blood supply (Mocé *et al.,* 2004; Argente *et al.,* 2008). Selection for litter size variability modifies early embryo development starting from 48 h of gestation, leading with a lower embryo development and a lower percentage of normal embryos in the line selected for increasing litter size variability. These results show negative relationships between litter size variability with embryonic development and survival in early stages of gestation.

Conclusions

Selection for litter size variability did not seem to affect ovulation rate. Nevertheless, there was a negative correlated response in early embryonic development and survival.

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Chapter 7

GENERAL DISCUSSION

In recent years, there has been an increasing interest in the genetic determination of the environmental variance (Morgante *et al.*, 2015; Sørensen *et al.*, 2015). In animal breeding, a decrease in environmental variance will increase the heritability, being particularly interesting for increasing the response to selection in low heritability traits, such as litter size (Argente *et al.,* 2010; Formoso-Rafferty *et al.,* 2016). Moreover, selection for reducing environmental variability can be useful for livestock industry, which is demanding a more homogeneous production (Mulder *et al*., 2008).

The animals object of this thesis come from two lines divergently selected by litter size environmental variability in rabbit. In this experiment, the use of complex models on environmental variability is avoided by directly selecting for this trait as an observed trait (Argente *et al*., 2014a). The selection criterion is based on phenotypic variance of litter size within female after correcting litter size for the effects of year-season and parity-lactation status. Genetic and permanent effects are common for all records of each female (Piles *et al.*, 2006), thus correcting litter size for systematic effects leaves only the residual random effect, and the phenotypic variance within female is a direct estimate of the environmental variability of litter size.

After seven generations of selection, the high (H) and the low variability (L) lines showed a difference of 1.19 kits^2 for environmental variability (Argente *et al.*, 2014a). Our hypothesis is that females can show a higher litter size variability due to high sensibility to stress and a lower disease resistance. In this regard, Argente *et al.* (2014b) found a lower immune response against pathogenic agents in females from the heterogeneous line, showing greater sensitivity to diseases than those from the homogeneous line. For this reason, three experiments were proposed to analyse the effect of selection for litter size variability in body condition and energy mobilization, such as biomarkers of animal welfare (Chapter 4), in litter size and its components (Chapter 5), and in early embryo survival and development (Chapter 6).

Stress has a negative effect on resource allocation and body condition (Elsasser *et al.,* 2000; Broom, 2008). Therefore, animal welfare can be measured by

the management of body reserves and energy mobilization at decisive moments in the production of females, i.e. mating (Castellini *et al*. 2006; Brecchia *et al*. 2006), delivery (Rebollar *et al*., 2011; Savietto *et al*., 2016) and early lactation (Quevedo *et al.*, 2006). Body condition is related to body fat reserves (review by Chilliard, 1993), and NEFA levels reflect essentially the breakdown of body fat reserves (Herdt, 2000). Firstly, we have studied the relationship between body reserves and energetic mobilization at mating, delivery and 10 d after delivery (Chapter 3). We have shown that body weight and perirenal fat thickness are good indicators of body reserves at mid-long term. They are necessary to measure at least in these three moments, because some of the correlations between measurements were not so high, therefore all of them give useful information about the energy balance in one reproductive cycle of the female. When the energetic mobilization is necessary to be measured, both NEFA before and after stimulating lipolysis were good indicators at short term. Both NEFAs showed high positive correlations when measured at the same time, but low correlations when measured at different times.

After seven generations of selection for litter size variability, there is some evidence that the H line had lower body fat than the L line at delivery (-0.16 mm) and 10 d after delivery (-0.17 mm), thus the line selected for homogeneity had a better body condition. At delivery, a time of high-energy demands, the L line exhibited a 30% higher concentration in NEFA after stimulating lipolysis than the H line, thus the homogenous line had a better mobilization of fat reserves. Therefore, the does from the L line exhibited better body condition and more efficient management in their body reserves under strong energetic demand, which suggests a higher health and welfare in animals from this line.

It is known that females under stress could reduce their productivity, measured as litter size (Zhao *et al*., 2013, in pigs; Zheng *et al.*, 2016, in mice). In our experiment, the H line showed 0.98 kits at second birth less than the L line. When litter size components were studied; the ovulation rate was similar in both lines. However, the number of implanted embryos were lower in the H line than in the L line (-1.48 embryos), as a consequence of a lower embryonic survival in the H line than in the L line (-0.09). Therefore, selection for residual litter size variability has a negative correlated response in number of implanted embryos and litter size. The

correlation between the mean and the variance of litter size has been the goal of several studies, with different results. A negative correlation has been detected in uterine capacity (Ibáñez-Escriche *et al*., 2008), a trait highly correlated with litter size (Argente *et al.,* 2000). Nevertheless, reanalysing the data after normalising the residuals, Yang *et al.* (2011) found a low positive relationship between both traits. Therefore, our results corroborate those found by Ibáñez-Escriche *et al*. (2008).

Early stages of pregnancy are more vulnerable to prenatal stress than later stages, usually due to changes in oviductal environment (Zheng *et al.,* 2016). For that, embryo survival and development were studied in our lines at 28 h, 48 h and 72 h of gestation. At 28 h of gestation, embryonic development and survival were similar in both lines, but at 48 h and 72 h of gestation, the line selected for increasing litter size variability showed lesser embryonic development and survival than the homogeneous line. It could be expected that higher percentage of normal and the advanced embryonic development in the L line could be due to favorable endocrine environment. The oviduct synthesizes and secretes many proteins in many species including the rabbit (Oliphant *et al.,* 1984), swine (Buhi and Alvarez, 2003), sheep and cattle (Nancarrow and Hill, 1995) which influence the gene expression of the developing embryos. Several proteins have an important role in embryo development and embryogenesis regulating (IGF1, Herrler *et al*., 1998; oviductine, Buhi, 2002; TIMP1, Hwang *et al*., 2000; uteroglobine, Riffo *et al*., 2007; leptin, Zerani *et al.*, 2004).

Our results are agreeing with that selection for litter size variability affects the environmental sensitivity of the females. Some indirect indicators, such as body condition and energy mobilization or productivity of the females, measured as litter size and its components, and embryos survival and development, showed that selection for homogeneity of litter size produced females with more capacity to adapt to stressful conditions than selection for heterogeneity of litter size. The pattern observed in this work might also be applied in other species in livestock production.

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Chapter 8

CONCLUSIONS

1. Body weight and perirenal fat thickness showed a high positive correlation between them, and they were related to body condition.

2. Non-esterified fatty acids, before ($NEFA_b$) and after lipolysis stimulation by isoproterenol ($NEFA_r$), exhibited a high positive correlation between them when they were measured at the same physiologic status.

3. NEFAs displayed low correlations with body condition measurements.

4. Body weight and perirenal fat thickness are related with fat reserves mobilization at mid-term, and NEFAs concentrations are related with the energy balance in the short-term.

5. The line selected to increase litter size variability showed lower fat thickness than the homogenous line at delivery (-0.16 mm, P = 0.86), and this difference remained at 10 d after delivery (-0.17 mm, P = 0.86). Therefore, the line selected for homogeneity had a better body condition.

6. The homogenous line exhibited 30% more concentration in $NEFA_r$ (P = 0.96) at delivery than the heterogeneous one, thus the homogenous line had a better mobilization of fat reserves.

7. The does selected for litter size homogeneity would be able to better deal with situations of high energy demand than does with higher litter size variability, indicating a more resiliency in these females.

8. Ovulation rate was similar in the lines selected to increase and decrease litter size variability.

9. The line selected for homogeneity in litter size showed more embryos at implantation (11.53 embryos vs. 10.20 embryos, P = 1.00) and higher embryonic survival than the heterogeneous line (0.87 vs. 0.78, P = 1.00).

10. A higher uterine overcrowding of embryos in the homogeneous line did not penalise fetal survival, and as a result, this line continued showing a greater number of kits born at birth than the heterogeneous one (7.94 kits vs. 7.16 kits, P = 0.96).

11. At 48 h of gestation, the heterogeneous line showed a 27% higher percentage of early morulae (P=0.94) and a 26% lower percentage of compacted morulae (P=0.93) than the homogeneous one, which is more advance embryonic development stage.

12. At 72 h of gestation, the line selected to increase litter size variability continued to show a lesser embryonic development than the homogeneous line (21.01% vs. 3.69% for percentage of early morulae, P=0.93; and 78.99% vs. 96.31% for percentage of compacted morulae and blastocysts, P=0.94).

13. A lower embryonic development in the heterogeneous line was related a lower embryonic survival than in the homogeneous line (-0.14, P=0.93) at 72 h of gestation.

14. Selection for higher litter size variability showed a negative correlated response in embryonic development and survival, which continued at implantation and posteriorly at birth for litter size.